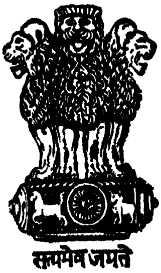

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DEPARTMENT
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Arnold Theiler died on the 24th July, 1936.

With his death closes a chapter in the history of veterinary science.

When Theiler came to South Africa in 1891 very little was known about the veterinary problems peculiar to the sub-continent. Tropical veterinary science was a subject as yet unborn. During the years which followed, Theiler, more than any other, helped to establish this branch of science and to create order where formerly there had been chaos. And before he died the majority of the problems which he encountered or formulated had, in the main, been solved; on others much light had been shed; to the solution or elucidation of almost all of them Theiler had contributed.

His publications cover wellnigh every field of veterinary science. In the early years he investigated rinderpest, lungsickness, horsesickness, nagana, equine piroplasmosis, and several other epizootic diseases of South African stock.

Then, after the Anglo-Boer war, he settled down in his laboratory at Daspoort; and the series of *Annual Reports of the Government Veterinary Bacteriologist* (1903-1910) published during those years bear testimony to his incredible capacity for work, his versatility and thoroughness, and his complete mastery of all the major veterinary problems of South Africa.

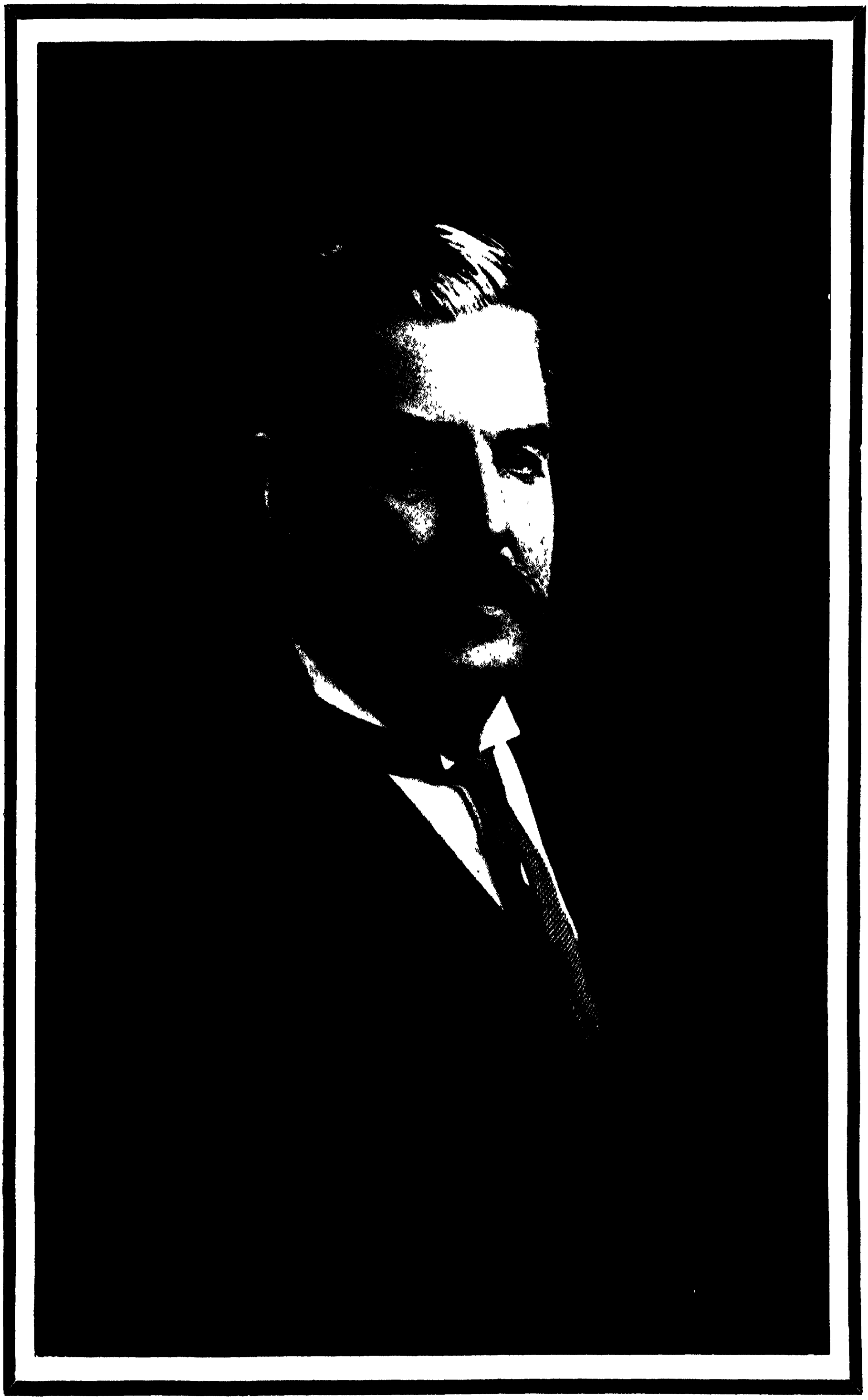
Thereafter, with his headquarters in the more spacious and well-equipped laboratories at Onderstepoort, the majority of his publications appeared in the fourteen voluminous *Annual Reports of the Director of Veterinary Research* (1911-1928). Here, again, the wealth of information contained in his scientific articles fills us with wonder and humility.

Even after his retiral from office in 1927, and indeed until his death, he continued his studies with unabated enthusiasm and brilliance, adding monumental contributions to the solution of the problem presented by the hitherto obscure group of osteodystrophic diseases.

Theiler's publications will for ever stand as a monument to the memory of a great man of science. His passing has cast a gloom over the Institute which he founded; but his spirit lives in the hearts and minds of those who had the privilege to know him and to work with him, and it is their ambition to emulate his high example.

The Onderstepoort Journal of Veterinary Science and Animal Industry is the continuation of the two series of *Annual Reports* which have been mentioned. May it follow in the paths trodden by Theiler and strive to uphold the lofty tradition which his labours have established.

P. J. DU TOIT.



Sir Arnold Theiler, K.C.M.G.

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ONDERSTEPOORT LABORATORIES,
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JULY, 1936.

List of Reports and Journals issued by the Director of the Onderstepoort Laboratories.

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903–4.*
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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1907–8.*
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Ninth and Tenth Reports of the Director of Veterinary Education and Research, April, 1923.
Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part I, September, 1926.
Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part II, January, 1927.
Thirteenth and Fourteenth Reports of the Director of Veterinary Education and Research, Parts I and II, October, 1928.
Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.
Sixteenth Report of the Director of Veterinary Services and Animal Industry, August, 1930.
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Eighteenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1932.
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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies on the Neurotropic Virus of Horsesickness V.

The Antigenic Response of Horses to Simultaneous Trivalent Immunization.

By R. A. ALEXANDER, Section of Protozoology and Virus Diseases, Onderstepoort.

It has been shown previously that there exists a plurality of antigenically different strains of horsesickness virus. Consideration of this important fact, in the light of results obtained with the neurotropic virus vaccine both in the laboratory and in the field during the season 1934-35, has shown that the problem of immunization has become one of the development of complete polyvalent immunity. On purely theoretical grounds it is reasonable to believe that this might be accomplished most satisfactorily by a series of injections each of which comprised a certain infecting dose of a different virus strain, but the practical difficulties attached to such a procedure are so vast that it is essential to limit the number of injections to a minimum. If possible immunization should be confined to a single subcutaneous injection of a mixture of the different strains. This is the procedure which has been adopted but it is necessary to record the antigenic response in horses as determined by in vitro serum neutralization tests and by in vivo immunity tests.

From time to time during the course of routine vaccine production by the method described (this journal) horses were injected with material taken at random from cold storage and kept at room temperature (±80° F.) for periods up to 14 days so as to approximate the conditions under which immunization was carried out in the field. Details of 5 such injections are the following:—

2/8/34.	Horse 20987.	10 cc. subcutaneously vaccine batch 8 prepared 24/7/34.
2/8/34.	Horse 20991.	10 cc. subcutaneously vaccine batch 10 prepared 1/8/34.
3/10/34.	Horse 20985.	10 cc. subcutaneously vaccine batch 24 prepared 27/9/34.
3/10/34.	Horse 20968.	10 cc. subcutaneously vaccine batch 27 prepared 3/10/34.
22/11/34.	Horse 20941.	10 cc. subcutaneously vaccine batch 40 prepared 20/10/34.

Each of the animals showed the anticipated mild febrile reaction to the vaccine and until required for bleeding were stabled in company with a number of susceptible horses, exercise being restricted to running in a bare dry paddock from 10 a.m. to 3 p.m. each day. During this period no cases of natural horsesickness occurred in the stables so that it is believed that between the time of injection and the time blood was tapped for the collection of serum the horses were not exposed to natural infection.

Since it has been shown that the antibody content of serum reaches a maximum approximately 6 months after the immunizing injection, neutralization tests were conducted with serum collected at this stage using the intra-cerebral protection test in mice as described previously (Alexander, 1935). The results are given in tabular form in Tables I, II and III on pages 11-13.

It will be noticed that unit volume of the serum dilutions were required to neutralize ±100 minimal infective doses of virus strain 449 but only ±50 M.I.D. of strains O and 464 B. In the tables no adjustment has been made as compensation for this slight variation in titre of the antigens since the results are so clear that this was considered both unnecessary and undesirable.

Consideration of the tables shows that each of the 5 horses developed antibodies against each of the three virus strains. There was some slight variation in the respective titres notably in the case of horse 20987 whose serum neutralized 449 virus only to a titre of $^1/_{16}$ but in the case of horsesickness even this low titre indicates that a solid immunity has been induced. The significance of these results will become clear if the tâbles are read in conjunction with those indicating the antigenic diffrences of the virus strains published previously (Alexander, 1935, pp. 369-371).

After the completion of the experiment the 5 horses used were exposed to natural horsesickness on the farm Kaalplaas which is notorious as a bad horsesickness farm. Throughout the season they remained in perfect health.

To confirm the results of this experiment by direct immunity test 8 other horses were immunized in a similar manner. Four weeks after immunization they were divided into 3 groups; group 1 (3 horses) received 10 cc. of virulent O virus intravenously; group 2 (3 horses) received 10 cc. of virulent 449 virus; group 3 (2 horses) received 10 cc. intravenously of a mixture of strains O and 449. There were no reactions. The virulence of the strains used for the immunity test was demonstrated subsequently by routine passage through susceptible horses all of which died. Unfortunately it was not possible to carry out an immunity test against strain 464 as this strain in its virulent form has been lost.

TABLE I.—*Neutralization of Virus Strain* 449.

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
5.2.35..........	Generation 181, strain 449.....	1 : 100	44444	44555	4455x	44560	55600	66000	00000	00000
29.3.35..........	,, ,, ...	,,	44444	44450	44555	45556	44550	60000	00000	00000
22.5.35..........	,, ,, ...	,,	44	50	45	45	44	70	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
5.2.35	Horse 20987, bled 21.1.35......	Days. 172	00	00	0000	*4500*	*5550*	4550	4550	4445	44	44
5.2.35	Horse 20991, bled 21.1.35......	172	00	0000	0000	0000	000x	5000	0000	*5566*	46	4x
29.3.35	Horse 20968, bled 27.3.35......	176	00	00	0000	0000	0000	6000	6000	*4455*	44	44
29.3.35	Horse 20985, bled 27.3.35......	176	00	00	0000	0000	6000	0000	*4600*	*4445*	45	55
22.5.35	Horse ——, bled 8.5.35.......	180	—	—	00	00	00	00	00	*44*	44	44

TABLE II.—*Neutralization of Virus Strain O.*

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/329.	1/640.
10.4.35	Generation strain O............	1 : 10	5666	5567	6660	6670	0000	x000	0000	0000
25.4.35	,, ,, ...	1 : 10	4556	5660	6660	5600	7000	0000	0000	0000
9.5.35	,, ,, ...	1 : 10	45	45	56	67	00	00	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
10.4.35	Horse 20987, bled 21.1.35......	Days. 172	0000	0000	0000	0000	6000	0000	7000	*5580*	—	—
10.4.35	Horse 20991, bled 21.1.35......	172	0000	0000	0000	0000	0000	*5700*	*5670*	6677	—	—
25.4.35	Horse 20968, bled 27.3.35......	176	0000	8000	7000	*6600*	*5670*	4660	5667	66	—	—
25.4.35	Horse 20985, bled 27.3.35......	176	0000	0000	0000	*5680*	*6700*	6678	5660	5677	—	—
9.5.35	Horse 20941, bled 8.5.35.......	180	00	00	00	00	*67*	77	57	56	—	—

TABLE III.—*Neutralization of Virus Strain 464 B.*

Antigen Titration.

Date.	Virus Antigen.	Conc.	Antigen Dilutions.							
			V.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
26.4.35	Generation 168, strain 464B.....	1 : 90	3444	4450	3450	4450	6000	0000	0000	0000
17.5.35	,, ,, ...	1 : 90	3444	4550	4444	4456	0000	0000	0000	0000
7.6.35	,, ,, ...	1 : 90	34	44	50	46	50	00	00	00

Neutralization Test.

Date.	Serum.	Interim.	Serum Dilutions.									
			1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.
		Days.										
26.4.35	Horse 20987, bled 21.1.35......	172	00	00	00	000x	4000	4000	*45*	44	45	44
26.4.35	Horse 20991, bled 21.1.35......	172	00	00	00	0000	0000	4000	*44*	40	44	44
17.5.35	Horse 20968, bled 27.3.35......	175	00	00	00	00	0000	0000	0000	000x	*4450*	56
17.5.35	Horse 20985, bled 27.3.35......	175	00	00	00	00	0000	4000	*4500*	*3456*	45	46
7.6.35	Horse 20941, bled 8.5.35.......	180	—	—	—	00	0000	0000	4000	*4555*	3450	4446

NOTE.—Conc. of Antigen = Serum saline dilution of stock emulsion.
In the tables the numbers indicate the day after injection on which the mice died.
0 = Survival for more than 9 days.
x = Death due to some cause other than horsesickness encephalitis usually injury at time of injection.
Thus, 450x means of 4 mice injected 1 died day 4, 1 died day 5, 1 died as a direct result of injection and 1 survived.

DISCUSSION.

It is not possible to generalize from the results obtained from so small a number of animals but it would appear that the simultaneous injection of 3 different strains of neurotropic attenuated virus results in the production of a solid immunity against each. In other words, there is no antagonistic action of one strain against another. This finding is the justification for issuing a vaccine which consists merely of a mixture of the different available strains diluted in such a way that the final product will contain not less than 100 infecting doses of each strain per dose of vaccine, and obviates the necessity of repeated injections in the field. Whether there will be a similar antigenic response when a greater number than 3 strains are included is a point which is being investigated at the moment and will be reported in due course.

SUMMARY.

By in vitro and in vivo methods it has been shown that the simultaneous injection of 3 strains of neurotropic horsesickness virus results in the production of a solid immunity against each.

REFERENCE.

ALEXANDER, R. A. (1935). Studies on the Neurotropic Virus of Horsesickness III. The Intracerebral Protection Test and its Application to the Study of Immunity. *Onderstepoort J. of Vet. Sci. and An Ind.*, Vol. 4, No. 2, pp. 349-377.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Horsesickness: Immunization of Horses and Mules in the Field during the Season 1934-1935 with a Description of the Technique of Preparation of Polyvalent Mouse Neurotropic Vaccine.

By R. A. ALEXANDER, W. O. NEITZ and P. J. DU TOIT,
Section of Protozoology and Virus Diseases, Onderstepoort.

RECOGNITION of the attenuation which accompanies neurotropic fixation of horsesickness virus strains in mice and guinea pigs (Alexander, 1933), has led to the development of a new method of immunization of horses and mules. The decision to introduce this method into general practice was taken after consideration of the promising results obtained under laboratory conditions (Alexander and Du Toit, 1934), and under field conditions on a small scale (Alexander and Van der Vijver, 1935). Up to the present time no detailed publication of the results obtained from the use of the commercial vaccine on large numbers of animals has appeared so that a review of the position after the horsesickness season 1934-35 will remedy this deficiency.

The results are being considered separately under two different headings, viz.: —

A. Immunization of remounts of the South African Police.

B. Immunization of other horses and mules.

This arbitrary subdivision has been made for several reasons. All the animals belonging to the Police were treated either by the Veterinary Officer of the Police personally (Major D. D. Morton, M.R.C.V.S.), or by his experienced farrier sergeants who could be relied upon faithfully to carry out all instructions and to use full aseptic precautions for all injections. In addition the conditions under which the Police animals are maintained made it possible to obtain in every instance a detailed report not only on the nature of the reaction to the vaccine but also on the subsequent history of the animal. This was essential as it was necessary to obtain reliable data on any possible adverse effect upon the performance of the horses as a result of immunization and to obtain an accurate diagnosis in the case of any reported breakdowns following exposure to natural infection. In the case of the remainder of the animals treated in spite of the fact that vaccine is issued to the veterinary profession only, many of the injections were carried out by stock inspectors under conditions which did not always permit of the observance of aseptic precautions or of the rational handling of a vaccine containing a living infective agent. In addition it was realized that it would be exceedingly difficult to obtain reliable reports on the reactions, and that an estimation of the degree of immunity conferred could only be approximate, since, in only a small percentage of reported breakdowns could the diagnosis be relied upon. Moreover, it was realized that a complete return from every user of the vaccine could never be hoped for

PREPARATION OF THE VACCINE.

The entire technique of vaccine preparation has been based upon that accumulated knowledge of the physical, chemical and biological properties of neurotropic virus contained in previous publications on the subject (Alexander, 1935).

Throughout the period under review the vaccine issued consisted of a mixture of 4 strains of virus which had been maintained " in pure culture " by separate serial passage in mice. The strains were those designated arbitrarily as O, 449, 464A and 464 B; the antigenic differences as determined by in vitro nutralization tests have been reported previously. In spite of some doubt as to the immunizing value of stain 464A it was included in the vaccine because repeated tests had shown it to have attained a safe level of attenuation for equines, and, as its injection apparently is innocuous, it was hoped that it might assist to immunize, at least partially, against some natural aberrant virus type at present not isolated; in other words it could do no harm but might be of value. For all practical purposes, however, it is considered that this vaccine was trivalent and not quadrivalent.

The 4 strains had been attenuated by the following number of intracerebral passages in mice: Strain O—143, strain 449—149, strains 464A and B—each 119.

The aim in vaccine production has been to turn out a bacteriologically sterile final product which would contain in 0·05 c.c. a minimum of 100 mouse infective doses of each of the strains. Since it has been shown that if 0·05 c.c. of a given emulsion contains approximately a single minimal infecting dose for mice then 10 c.c. will contain approximately 1 M.I.D. for horses, the routine dose was fixed at 10 c.c. given subcutaneously so that each dose of vaccine would contain not less than 100 M.I.D.'s of each strain. This was considered to be a perfectly safe margin and has been justified by the results of the vivo immunity tests and the in vitro neutralization tests published separately (Alexander, 1936, this journal).

In planning the production of a batch of vaccine the abovementioned points have been borne in mind together with the knowledge that—

(1) the different strains of virus produce in mice a disease characterized by appreciably different periods of incubation and course;

(2) there is a rapid increase in the virus titre of the brains of infected mice in the later stages of the disease, i.e. in that short period from the appearance of symptoms up to the time of death;

(3) rapid and easy removal of infective brains may be assured only by using those mice which are destroyed in extremis by ether or other anaesthesia and not mice which are found lying dead in the boxes. Brains from the latter frequently show advanced autolysis, usually are removed intact with considerable difficulty, and may have been invaded by bacterial contaminants.

Consequently the following routine technique for commercial vaccine preparation has been adopted:—

Day 1—in the afternoon mice injected with standard emulsion* of virus strain O (about 30 mice).

Day 2—in the afternoon mice injected with strain 449 (about 15 mice).

Day 3—in the morning mice injected with strains 464A and B (about 15 mice each strain).

Day 6—on arrival in the morning 2 or 3 mice of each strain will be found dead and should be discarded. The majority of the remainder will be in extremis; these are etherized, the brains removed with aseptic precautions as quickly as possible and placed in sterile 50 c.c. centrifuge tubes fitted with corks, 3 entire brains being placed in each tube. In the afternoon the remainder of the mice will or should be in extremis and the brains are removed as before. All the tubes are stored upright in the freezing chamber of a refrigerator until required. This freezing facilitates disintegration and subsequent emulsification.

Day 7—approximately 50 c.c. of 10 per cent. normal serum—saline is added to 7 tubes of strain O, and 3 each of strains 449, 464A and B. A coarse emulsion of each is made by drawing the material into and forcing it out of sterile Agla syringes. The tubes are returned overnight to the freezing chamber of the refrigerator.

Day 8—the virus emulsions are rapidly thawed in an incubator at 37° C. and a fine emulsion prepared with sterile precautions as above. The tubes are centrifuged at about 3,500 revolutions per minute for 10 minutes and the supernatant fluid decanted into sterile containers of suitable capacity (120 c.c. bottles fitted with cotton wool stoppers were used) the strains being kept separate so as to make replacement simple should any accident occur. From each container 0·5 c.c. of emulsion is seeded onto large agar slants for bacterial sterility test by incubation for at least 48 hours.

Day 9—providing the sterility test is satisfactory the virus emulsions are added to 10 per cent. serum saline, the volume adjusted to 6 litres and 2 per cent. ether added as a preservative. After thorough shaking to ensure mixing of the virus strains as well as solution of the ether the vaccine is bottled, corked and sealed. The final bottled product is incubated overnight at 37° C., a precedure which greatly enhances the bactericidal action of the ether without affecting the virus titre and then stored in a refrigerator at 4° C. until required after a second sterility test has been carried out on 1 or 2 bottles selected at random. The virus titre does not decrease appreciably on storage at ±4° C. for up to 2 months, but does diminish fairly rapidly at room temperature. Consequently it is stipulated that the injections must be carried out within 14 days of issue from the laboratory. This has been shown to be an adequate and a safe margin.

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* Standard virus emulsion constantly in use is a 5 per cent. emulsion of infective mouse brains in a 10 per cent. normal serum saline.

It will be noted that the original virus emulsions of strains 449, 464A and B were diluted approximately 1:40. At this dilution 0·05 c.c. always contains at least 100 mouse infective doses; usually the titre more nearly approximates 250 M.I.D. Similarly prepared emulsions of neurotropic virus strain O invariably contain rather less than half these titres and this explains the use of 21 brains instead of 9. On this basis 48 mouse brains are required for the production of 600 doses of vaccine, i.e. 12·5 doses per brain.

At first sight the entire procedure may appear to be complicated but in actual practise that is not the case more particularly when the procedure is adapted to the preparation of at least one batch of vaccine every day. The quantities selected naturally may be varied to meet particular requirements but they were chosen as being most suitable to the equipment available. Moreover 6 litres of vaccine (i.e. 600 doses) per day was found to be sufficient to meet all demands.

In considering the technique described it may seem somewhat unnecessary to recommend that the injection of one strain of virus should be carried out in the afternoon while another strain should be injected in the morning, but the nature of the disease produced by a fixed neurotropic virus is so constant that a considerable experience with the production of some 90,000 doses of vaccine has shown the digerential treatment to be essential for maximum virus production together with economy in the numbers of mice used.

Mention must also be made of the bacterial sterility of the vaccine prepared. From the number of manipulations described it would not be unreasonable to expect that a number of vaccine batches would show a fairly heavy contamination, which could not be controlled by ether whose action in 2 per cent. concentration is bacteriostatic rather than bactericidal. Again experience has shown that this is not the case. Apparently any contaminants picked up during removal of the brains or introduced during any process requiring removal of the cork from the tubes become entangled with the fine suspended brain particles of the crude emulsions and are carried down mechanically during centrifugation. This is the only explanation that can be offered for the rarity of finding an infected emulsion. Out of about 150 batches of vaccine prepared only 2 have been discarded on account of infection; examination of records has shown that on both occasions the centrifuged emulsions had been left in the refrigerator overnight before being decanted, thus permitting diffusion of organisms from the deposit. Incidentally consideration of the results of immunization will show that abscessation at the site of injection has not been a complicating factor.

At this point it is desirable to emphasize the necessity for particular attention to uniformity of technique if a final product of constant potency is to be obtained regularly. Mice of the same strain, age, and weight should be used (for this work mice about 12 weeks old have been found most satisfactory) injections should be carried out at the same time each day, and the infectivity and dose of inoculum should not vary. If strict uniformity of technique is adopted it will be found that the virus titre of the final emulsions will vary within exceedingly narrow limits and this will minimize the number of potency tests necessary.

The actual production of the vaccine would be simplified by the discovery of some substance to replace the serum of the serum saline vehicle to counteract the virucidal action of saline alone. Preferably such a substance should be heat stable so as to enable sterilization by autoclaving to be carried out. Furthermore extensive research is necessary to bring to light some preservative more efficient than ether, but it should not have the selective bacterial action of many of the dyes and of course must be non-irritant and non-virucidal over a wide range of temperature.

A. THE IMMUNIZATION OF POLICE REMOUNTS.

A total of 1,815 horses were immunized over an area comprising practically the entire Union excluding the south-west Cape. In the larger centres the horses were maintained in the regular depots. In the outlying districts they were concentrated at suitable points for injection and then returned immediately to their posts. The routine procedure was to rest horses for 21 days after injection, then to put them on light patrol duty for 14 days before gradually bringing them back to full work. During the period of rest rations were reduced to the requirements for maintenance only, green feed being augmented where possible, and exercise was limited to a minimum.

Reactions.—In approximately 100 cases temperature records were kept, in the remainder observations were recorded on the general habitus of the horses. It became apparent that there is a considerable variation in the reaction produced in different animals maintained under identical conditions. Some horses show no demonstrable reaction whatever but in the majority a febrile and in some cases a general systemic reaction commences on approximately the 6th day and lasts for 3-5 days. During the period of pyrexia the afternoon temperature may rise as high as 105-106° but the morning temperature is seldom above 101. This diurnal fluctuation in temperature may be considered typical but occasionally there is a period of continuous fever. Usually return to normal follows the decrease in the afternoon temperature exacerbations. A remarkable fact is the observation that in spite of a fairly severe fever there is little or no constitutional disturbance other than that directly associated with hyperthermia (e.g. slight pulse acceleration and polypnoea). As a rule horses do not go off their feed and only a careful examination and a record of the afternoon temperature will indicate any deviation from normal. In only 1 or 2 cases was the period of convalescence, following the most severe reactions, prolonged beyond the 21st day and the percentage of cases showing edema of the supraorbital fossae (dikkop), noticeable on about the 12th day, was less than 0·5 per cent.; mortality as a direct result of immunization was nil. In fact the position may be summarized by saying that in approximately 75 per cent. of cases the reaction in fully susceptible animals is so mild as to be detectable only by an experienced and competent observer. Animals which had been immunized previously either by the serum virus method, or by the neurotropic virus method even by the injection of only a single attenuated strain showed no reaction whatever to subsequent treatment.

After Effects.

As stated above an accurate record of the subsequent history of every horse was kept, the record including the amount and nature of the work carried out. In almost every instance the allocation of mounts remained unchanged prior and subsequent to immunization so that the opinion of the rider on any change in the performance of his mount could be obtained. In only one instance did a report show that a horse apparently had become sluggish some months after return to work. Veterinary inspection failed to connect this with horsesickness immunization and the animal picked up in condition rapidly after anthelminthic treatment.

Again the position may be summarized by saying that no harmful after effects were encountered, observations being made on horses engaged in all types of work from light patrol duty to the strenuous demands of daily attendance in the school of equitation at the Depot. No cases of staggers (acute liver atrophy) were encountered.

Immunity.

On exposure to natural infection during the horsesickness season 12 immunized horses (0·66 per cent.) contracted the disease. Of these 10 (0·55 per cent.) died and 2 (0·11 per cent.) recovered. Full details of these cases will be found in the appendix at the end.

In the two cases which recovered a diagnosis was made from the appearance of a typical dikkop; it is uncertain whether other cases of horsesickness fever unaccompanied by dikkop and hence undiagnosed aetiologically occurred. In the fatal cases a diagnosis was made either from the isolation of virus from blood collected immediately prior to death, or from consideration of evidence submitted to a board of inquiry. There is no reason to believe that an accurate diagnosis was not made in each case and it is not possible for any death not to have been reported.

Consideration of the appendix will show that 6 of the breakdowns occurred in the eastern and north-eastern Transvaal, 3 in the Natal-Zululand area, 2 in the Vryburg area and 1 in the Pretoria District. From the Natal and Transvaal cases 3 strains of virus have been isolated. These have been fixed neurotropically in mice and preliminary work has shown that they differ antigenically from the strains incorporated in the vaccine. A detailed report on the antigenic inter-relationship will form the subject of a future publication. From the one Pretoria and two Vryburg breakdowns unfortunately no strain of virus was isolated.

It is of interest to note that 6 of the 12 horses under consideration had been immunized by the serum virus method some time prior to reimmunization by the neurotropic virus method and that 1 horse had received 2 injections of neurotropic vaccine. Further in almost every instance there is a history of the horse dying somewhat suddenly, either immediately after return from patrol duty or after being noticed sick for the first time when actually on the road. The significance of this observation is not quite clear but it indicates the harmful effect of work on an immune animal undergoing either a definite or abortive reaction as a result of natural infection. This conclusion is supported by the finding that the strains of virus isolated proved fatal to susceptible animals in the laboratory but produced merely severe febrile and systemic reactions in horses that had been immunized previously with the routine vaccine but were maintained at rest in the stable during the entire period of the immunity test.

B. IMMUNIZATION OF HORSES AND MULES, OTHER THAN POLICE REMOUNTS.

This group comprises all types of horses from nondescript hacks to schooled polo-ponies and from purebred representatives of the heavy draft breeds to valuable Thoroughbred stud stock. In addition a large number of mules were treated but unfortunately when the returns were submitted a differentiation between horses and mules was not made in every instance, so that it may be stated only, that the number of mules immunized was not less than 1,524. As far as it is possible to do so the results are tabulated in the accompanying Table I

[NOTE.—These figures were compiled from reports submitted by Government Veterinary Officers for the districts under their control. These districts do not correspond with the magisterial districts, so that in some cases the classification of the districts may appear to be at fault.]

It must be conceded at the outset that these figures cannot be regarded as being other than merely approximate. For instance, according to the reports received a total of 28,659 horses and mules were immunized, whereas examination of the record of issues made shows that 31,416 doses of vaccine were supplied to meet orders received. Thus there is no record of the results obtained from the use of 2,757 doses of vaccine. It has not been possible to ascertain exactly how the discrepancy has occurred, but it is in part made up by the omission of 1,057 animals in the Piet Retief area from which no details are available. This omission is regrettable because the district is situated in that portion of the Union where the percentage of breakdowns might be anticipated to be not lower than that reported from Vryheid, namely 2·60 per cent.

Reaction to Immunization.—On two occasions it was reported that severe abscessation and local phlegmosis had occurred at the site of injection. The total number of animals involved was 8, but in each case it was determined that of over 1,000 horses injected with the same batches of vaccine no others had shown any local reaction. It must be concluded therefore that pyogenic infection had gained entrance from the use of contaminated needles and syringes and that the vaccine itself was not the primary cause.

TABLE I.

Results of Horsesickness Immunization, 1934-35.

Horses and Mules other than Police Remounts.

Province and District.	Number of Animals Immunized.			Number of Breakdowns in Immunity.					
	Horses.	Mules.	Total.	Deaths.	Per Cent.	Recovery.	Per Cent.	Total.	Per Cent.
A.—CAPE.									
Mafeking........	—	—	2,653	24	0·90	26	0·98	50	1·88
Vryburg.........	—	—	1,193	1	0·09	0	0	1	0·09
Kimberley.......	734	10	744	0	0	0	0	0	0
De Aar..........	—	—	822	0	0	0	0	0	0
Middelburg.......	—	—	180	0	0	0	0	0	0
Aliwal North.....	—	—	12	0	0	0	0	0	0
Capetown........	—	—	58	0	0	0	0	0	0
Port Elizabeth...	244	377	621	0	0	0	0	0	0
East London.....	—	—	209	0	0	0	0	0	0
Grahamstown....	—	—	343	0	0	0	0	0	0
Umtata..........	—	—	300	0	0	0	0	0	0
Flagstaff.........	—	—	129	0	0	0	0	0	0
Kokstad.........	—	—	394	0	0	0	0	0	0
B.—ORANGE FREE STATE.									
Kroonstad.......	1,030	252	1,282	0	0	0	0	0	0
Bloemfontein.....	295	36	331	0	0	0	0	0	0
C.—TRANSVAAL.									
Highveld.									
Ermelo........	—	—	2,501	5	0·20	2	0·08	7	0·28
Potchefstroom..	1,890	51	1,941	5	0·26	1	0·05	6	0·31
Johannesburg..	376	8	384	0	0	0	0	0	0
Middelburg.....	2,094	12	2,106	16	0·76	2	0·09	18	0·85
Northern.									
Pretoria.......	1,977	357	2,334	24	1·03	10	0·43	34	1·46
Potgietersrust..	—	—	464	5	1·08	1	0·22	6	1·30
Pietersburg....	381	42	423	8	1·89	7	1·65	15	3·54
Louis Trichardt	—	—	237	5	2·11	0	0	5	2·11
Rustenburg....	—	—	237	0	0	1	0·42	1	0·42
Eastern.									
Barberton......	—	—	171	8	4·68	1	0·59	9	5·27
D.—NATAL.									
Highveld and Middle Veld.									
Dundee........	1,419	57	1,476	6	0·41	3	0·25	9	0·66
Estcourt.......	1,041	54	1,095	1	0·10	0	0	1	0·10
Ladysmith.....	955	16	971	1	0·1	0	0	1	0·10
Greytown......	601	16	617	1	0·17	2	0·33	3	0·50
Pietermaritzburg........	203	48	251	0	0	0	0	0	0
Ixopo.........	208	44	252	0	0	0	0	0	0
Low Veld.									
Vryheid.......	1,926	116	2,042	32	1·57	10	0·49	42	2·06
Nongoma......	—	—	57	4	7·02	9	12·28	11	19·30
Eshowe........	109	28	137	10	7·30	5	3·65	15	10·95
Durban........	—	—	203	0	0	0	0	0	0
Port Shepstone	—	—	82	1	1·22	0	0	1	1·22
E.—SOUTH WEST AFRICA	—	—	943	0	0	0	0	0	0
F.—SWAZILAND..	—	—	217	5	2·30	0	0	5	2·30
G.—S. RHODESIA	—	—	247	3	1·21	4	1·62	7	1·83
TOTAL.........	—	1,524	28,659	165	0·58	82	0·29	247	0·87

Several reports were received from farmers that individual animals had died within 48 hours of injection. Obviously the deaths could not be attributed to the action of the vaccine-virus to that it is believed that these reports represented coincidences unconnected with immunization. On the other hand it is necessary to detail the histories of 3 horses which died under circumstances which cannot exclude the vaccine from all blame with certainty.

1. District Estcourt, Natal—horse injected 29/11/34; noticed to be dull on 6th day; on 8th day fever and swelling of the supraorbital fossa; died on 9th day. After post-mortem examination an aetiological diagnosis of Dikkop Horsesickness was made but this could not be confirmed by histological examination of specimens submitted and no virus was isolated from the blood.

2. District Klerksdorp, Transvaal—horse injected 5/12/34; noticed to be dull and sluggish on 9th day; died on 12th day after showing symptoms described by the owner as being typically those of dunkop horsesickness. No opportunity of confirming or refuting this diagnosis was available.

3. District Machadodorp, Transvaal—it was merely reported that a horse had died on the 13th day after injection after showing typical symptoms of horsesickness.

Although these reports are vague and the diagnosis in each case was unconfirmed it is considered that the reaction to the vaccine may have been a factor contributing to the death of the animal. In spite of this it is merely necessary to point out that the death of 3 horses out of a total of 28,659 represents a percentage mortality which may be disregarded.

One other adverse report merits attention. The Government Veterinary Officer in Kimberley was advised that out of a troop of 40 horses in the Spytfontein District 1 was noticed decidedly ill on the 5th day after injection but appeared to have recovered by the 8th day. On the 7th day 2 mares and a gelding suddenly "went mad". After carreering wildly about a paddock they damaged themselves by charging into fences or trees and either succumbed to their injuries or had to be destroyed. Veterinary inspection of the remainder on the 9th day showed only a single mare undergoing a typical but severe horsesickness reaction, and careful examination of the pasture for the presence of poisonous plants yielded negative results. This report would appear to indicate neurotropism of the vaccine virus with involvement of the central nervous system possibly in peculiarly susceptible animals, but in the absence of corroborative evidence it must remain for the present an isolated and unconfirmed occurrence.

For the rest a careful examination of the returns shows that a large number of owners were disappointed at the absence of any clinical reaction because they believed, that without at least a definite horsesickness fever, no immunity could result; a further large number reported a mild febrile reaction occurring some time between the 6th and 13th day after injection; less that 3 per cent. reported severe febrile reactions and only rarely is mention made of the incidence of supraorbital edema (dikkop).

The horses in question were maintained under a wide variety of conditions. In a limited number of cases the hygiene, feeding, and general management left nothing to be desired but it was a fairly general practise to turn out injected horses under every variety of climatic conditions to fend for themselves on poor quality grass until the completion of the prescribed period of 21 days rest. Many horses were worked for at least 5 days after injection and were returned to work within a fortnight, others were worked continuously and yet there appeared to be no accidents. These practises cannot be condemned too strongly but the attenuation of the vaccine virus has attained such a degree that apparently it may be used with impunity under all conditions of farming in South Africa.

It is necessary to make no differentiation with regard to the immunization of mules. But, from two separate sources, it has been reported that a total of 5 mules showed a complete or partial blindness which developed progressively from about the 28th day after injection. All attempts to procure these animals for the purpose of carrying out a detailed examination were unsuccessful so that the entire question of neurotropism of the virus in mules must remain an open one pending the collection of additional data on a larger number of cases. In the laboratory a similar condition has not been encountered.

After-effects.—In one or two instances owners expressed the opinion that individual animals appeared to be sluggish for some months after treatment. On the other hand the observations of experienced horsemen, and of the members of recognized polo clubs playing immunized ponies regularly, makes it clear that the general concensus of opinion is that unpleasant sequelae need not be feared.

Immunity.—At the end of the horsesickness season it was reported that the immunity of 247 (0·87 per cent.) of immunized horses and mules had been broken down on exposure to natural infection. Of this number 165 (66·7 per cent.) died and 82 (33·3 per cent) reacted severely but recovered. It will be noticed that these figures correspond fairly closely with hose obtained with the police horses.

The heaviest mortality was experienced in the Natal-Zululand, Northern and Eastern Transvaal areas. In Nongoma the breakdowns amounted to 19·30 per cent. out of a total of only 57 immunized, in Eshowe 10·95 per cent. out of 137. Vryheid, Barberton, Louis Trichardt and Pietersburg showed breakdowns varying from 2 to 5·27 per cent. Blood was collected from a number of these animals immediately before death and preliminary work by in vivo methods has shown that the virus strains isolated are antigenically similar to the aberrant strains isolated from the police horses.

In the Mafeking area 1·88 per cent. of 2,653 immunized animals proved to be inadequately protected. Unfortunately only a single virus sample was collected from these animals. The virus isolated produced no reaction in animals immunized against the vaccine plus aberrant police strains, but from this single strain it is not possible to express any opinion as to the possibility of other antigenically dissimilar strains occurring over this wide area. This point is being borne in mind because of the great difference in climatic conditions between the eastern and western portions of the Union, a factor which might favour the existence of yet another virus type.

From a statistical point of view it is admitted that these figures have very little value. In the first place the diagnosis of horsesickness usually was made by laymen who are prone to believe that every immunized horse which dies during the late summer months must have died of horsesickness. It is surmized however that the number of incorrect diagnoses approximately would counterbalance the number of breakdowns which were not reported. In the second place the incidence of horsesickness among non-immunized animals maintained under similar conditions could not be ascertained, thus from an experimental and comparative point of view the fate of the all important controls cannot be recorded.

DISCUSSION.

Consideration of the results reported above immediately emphasizes one important point, namely, that the neurotropic virus method of immunization of horses and mules may be practised with perfect safety. Out of 1815 police animals treated there was not a single death attributable to the use of the vaccine. Out of at least 28,659 other animals in general practise there have been recorded 3 deaths for which there is some possibility of the vaccine itself being responsible. This percentage is negligible and marks a considerable advance over the results obtained with the serum virus method where a mortality of 10 per cent. as a direct result of immunization has been recorded on several occasions.

Every effort has been made to determine the possible danger of neurotropism of the attenuated virus strains for equines. If this danger does exist it is so remote as to be almost negligible, as indicated be the experience in the field with over 30,000 equines and by direct and indirect methods in the laboratory with a limited number of animals. An unqualified statement that there is no danger of serious nervous complications cannot be advanced owing to the reported incidence of 5 cases of blindness in mules. This entire question, together with the affinity of the neurotropic virus for the nervous system of mules and donkeys, is being investigated further and will be reported in due course. At least there appears to be no necessity to complicate the entire method of immunization by developing a technique which will include the simultaneous use of hyperimmune serum, as has been found to be essential in the analogous case of Yellow Fever in man.

With regard to any remote ill effect of immunization it may be stated that up to the present time no unpleasant sequelae have been encountered. Certainly no permanent physical disability such as myocardial weakness or persistent slugglishness need be feared. The incidence of staggers (acute liver atrophy) may be cited with some justification as a complicating factor of great importance in the serum-virus method, but up to the present time no cases have occurred which could incriminate the present vaccine. Since the aetiology of this condition remains obscure it is undesirable to do more than record this observation.

The figures indicating the degree of immunity to natural infection are not entirely satisfactory because the season under review must be regarded as a mild one from the point of view of natural incidence of the disease. A recorded percentage of breakdowns in the case of police horses of 0·66 per cent. and in the case of other equines of 0·87 per cent. quite probably errs on the side of flattering the vaccine. This statement is supported by the fact that in some areas such as Nongoma, Eshowe, Vryheid, where every season must be regarded as a bad horsesickness season the mortality was as high as 19·30 per cent though the number of horses immunized was small. (Nongoma 10·30 per cent. of 57. Eshowe 10·95 per cent. of 137. Vryheid 2·06 per cent. of 2,042.) However it must be remembered that from this geographically and climatically similar tract of country there was isolated at least one strain of virus which differed antigenically from those which were available for atteuuation. Natural infection with this strain probably was responsible for the adverse figures. On the other hand it is known that in some parts of the country the mortality from horsesickness amongst susceptible horses was high in spite of the usual prophylactic measures to prevent infection and yet the percentage breakdowns amongst immunized animals was less than 0·5 per cent. These observations, regarded in the light of the laboratory experience which has shown definitely that injection of a certain infecting dose of attenuated virus is followed by a solid immunity to the homologous strain, encourage the belief that the problem has become one of the development of complete polyvalency. Whether this is possible is a question which cannot be anwersed at present from the limited data available.

A number of additional problems possibly of minor importance await solution. For instance from the breeders point of view it is necessary to determine the effect of immunization on fecundity; the effect on susceptible pregnant mares at various stages of pregnancy; whether foals from immune dams are born with a passive resistance and if so the duration of that resistance; the youngest age at which immunization is both safe and efficacious. Then, from a general point of view, the duration of polyvalent immunity is a matter of importance. With the exception of the latter these are problems which do not lend themselves readily to solution by direct experimental methods in the absence of unrestricted. access to a stud of considerable magnitude.

In conclusion it may be stated that neurotropic virus vaccine method of immunization has been attended by the most gratifying results. It is admitted freely that the method still awaits perfection, but a sound foundation has been laid on which to build.

SUMMARY.

1. Details of the method adopted for the preparation of the vaccine are given.

2. The results obtained in the field during the season 1934-35 with the immunization of 1,815 police remounts and 28,659 other horses and mules are discussed.

Of the police horses there was no mortality as a direct result of immunization, no adverse after effects were noted, and the immunity of 12 was broken down on exposure to natural infection. Of those 10 died and 2 recovered.

Of the other horses and mules there were 3 deaths for which the vaccine may have been responsible or to which it may have contributed. On the whole the reactions were exceedingly mild and there were no adverse after effects. The immunity of 247 (0·87 per cent.) was broken down on exposure to natural infection. Of these 165 (0·58 per cent.) died and 82 (0·29 per cent.) recovered.

3. From the police breakdowns 3 strains of virus were isolated and have been fixed neurotropically in mice. Their antigenic interrelationship is being worked out.

4. In mules 5 cases of blindness following immunization have been reported.

5. The problem of immunization by the neurotropic virus method is discussed.

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II. Some Chemical and Physical Properties.

III. The Intracerebral Protection Test and its Application to the Study of Immunity.

IV. The Pathogenesis in Horses.

Onderstepoort Journ. of Vet. Sc. and An. Ind., Vol. 4, No. 2, pp. 291-388.

APPENDIX.

Details of Cases Representing Breakdown of Immunity amongst Police Horses.

1. Horse T.387, stationed at Nelspruit, E. Transvaal. 19.3.34, immunized with neurotropic vaccine in Pretoria Depot. 23.8.34, immunized Nelspruit with neurotropic vaccine after transfer to that district. 19.1.35 reported sick, showing symptoms of Dikkop horsesickness. Died 21.1.35. Aetiological diagnosis, horsesickness.

2. Horse E.166, stationed at Ruimte, E. Transvaal. 21.1.35, sent to Schildpadfontein on duty; on arrival noticed to be off colour and sluggish; from 22.1.35 to 27.1.35 showed symptoms of fever up to 105° F., diagonsed as horsesickness; recovery uninterrupted and returned to work on 2.2.35.

3. Horse H.199, stationed at Bushbuckridge, Eastern Transvaal; purchased 1933, and immunized by serum virus method prior to purchase; 6.7.34, immunized by neurotropic vaccine. 25.1.35, returned from a 4-day patrol. 26.1.35, noticed sick. 28.1.35, died; aetiological diagnosis, horsesickness.

4. Horse T.459, stationed at Eshowe, Natal. 24.4.34, immunized neurotropic vaccine. 1.9.34, immunized neurotropic vaccine. 2.2.25, noticed sick. 23.2.35, died. Diagnosis, horsesickness. Blood sample collected before death.

5. Horse E.517, stationed at Nylstroom. 29.11.28, immunized by serum virus method. 7.8.34, immunized neurotropic vaccine. 4.3.35, on 25-mile patrol. 5 3.35, noticed sick and on examination showed dikkop. 7.3.35, died. Aetiological diagnosis, dikkop horsesickness. Blood sample collected before death.

6. Horse T.737, stationed at Schoonoord, District Middelburg, Transvaal. 20.7.34, immunized neurotropic vaccine. 1.4.35, ridden from Schoonoord to Pakwani, left at 7 a.m. and arrived at 3 p.m.; started return journey following afternoon; after travelling 3 miles, noticed sluggish and immediately led back to Pokwani. 3.4.35, died. Aetiological diagnosis, dikkop horsesickness.

7. Horse H.960, stationed at Brits. 4.4.32, purchased after immunization by serum virus. 28.8.34, immunized neurotropic vaccine. 8.4.35, started on patrol at 7 a.m.; at noon horse noticed sluggish, immediately off-saddled and allowed to return to camp at its own gait. 9.4.35, died. Diagnosis, dikkop horsesickness.

8. Horse H.927, stationed at Schwayane cordon, Vryburg District. 4.4.32, purchased after serum virus immunization. 29.4.34, immunized neurotropic vaccine. 21.5.35, noticed sick, showing dikkop. 22.5.35, general condition brighter but marked oedema of head and throat; during the day struggled to break loose, and after breaking the leather dropped dead.

9. Horse B.280, stationed at Ntambanana, District Eshowe, Natal. 29.8.34, immunized neurotropic vaccine. 28.4.35, left at 2 a.m. for Empangeni, being led; when about 5 miles from Empangeni, commenced purging and appeared dull and distressed. Continued slowly on journey, arriving at 7.15; died 2 hours later. Horse did not feed or drink during the journey. Diagnosis, horsesickness.

10. Horse H.957, stationed at Matubatuba, District Eshowe, Natal. 27.6.33, immunized by serum virus method. 30.8.34, immunized neurotropic vaccine. 18.4.35, noticed sick when grazing in a paddock. 19.4.35, died. Diagnosis, horsesickness.

1. Horse H.913, stationed at Pietersburg, Transvaal. 4.4.32, immunized serum virus. 9.7.34, immunized neurotropic vaccine. 2.7.35, went on patrol and completed 30 miles, before return next day, being noticed sluggish before reaching camp. 4.7.35, died. Diagnosis, dikkop horsesickness.

12. Horse E.336, stationed at Honeyskop, District Taungs, Transvaal. 29.9.34, immunized neurotropic vaccine. 14.4.35, sick and subsequently showed dikkop. Recovered.

Section II.

Parasitology.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Notes on Species of Trichodectidae with Descriptions of New Genera and Species.

By G. A. H. BEDFORD, Section of Parasitology, Onderstepoort.

MOST of the material reported on in this paper from Procaviidae was collected by Mr. Gordon B. Thompson and the writer of skins in the British Museum, and the numbers recorded between brackets after their hosts refer to the numbers of the skins in the museum. For permission to examine the skins I am deeply indebted to Mr. Martin A. C. Hinton, F.R.S.

I also wish to express my thanks to the following for sending me material:—Mr. Gordon B. Thompson of the British Museum; Mr. G. H. E. Hopkins for species from Uganda and Kenya Colony, and Dr. G. Martinaglia for material collected off animals in the Zoological Gardens, Johannesburg. To Mr. G. B. Thompson I am further indebted for kindly sending me copies of both Gervais' and Rudow's original descriptions and tracings of their drawings of species described by them found on goats.

Genus PROCAVICOLA Bedford.

Procavicola univirgata (Neumann).

Previous records.—From "*Hyrax*" sp., Congo (Neumann, 1913); *Dendrohyrax* sp., Berlin Museum (Stobbe, 1913); *D. adolfi-friederici*, Belgian Congo (Ferris, 1930); *D. arborea*, Port St. Johns, Cape Province (Bedford, 1932).

Additional records.—*Dendrohyrax arborea*, Port Alfred, Cape Province, 1933 (coll. R. F. Lawrence); *D. adolfi-friederici*, Mount Muhavura, south-west of Kigezi, Uganda, 9th Sept., 1929 (Brit. Mus. No. 30.8.1.54); *D. crawshayi*, Kinangop, Kenya Colony (Brit. Mus. No. 3.4.4.4.); *D. scheelei*, Ukeke District, Tanganyika Territory, 8th Dec., 1910 (Brit. Mus. No. 11.4.23.2); *D. stuhlmanni*, Burumba, Ankole, Uganda, Aug., 1903 (Brit. Mus. No. 4.2.6.32); *D. bocagei*, Benguella, Anboim District, Angola, 27th April, 1934 (coll. K. Jordan) and *D. angolensis*, Congulu, Amboim District, Angola (coll. K. Jordan).

Procavicola neumanni (Stobbe).

Previous records.—From *Dendrohyrax* sp., Berlin Museum (Stobbe, 1913); as *Trichodectes sternatus* from *Dendrohyrax adolfi-friederici*, Belgian Congo (Ferris, 1930); *D. arborea*, Port St. Johns, Cape Province (Bedford, 1932).

Additional records.—*Dendrohyrax arborea*, Port Alfred, Cape Province, 1933 (coll. R. F. Lawrence); *D. adolfi-friederici*, between Masisi and Labutsi (Brit. Mus. No. 28.1.30.29); *D. crawshayi*, Kinangop, Kenya Colony (Brit. Mus. No. 3.4.4.4) and Tuthu, Kenya Colony (Brit. Mus. No. 3.4.4.6); *D. scheelei*, Ukeke District, Tanganyika Territory, 8th Dec., 1910 (Brit. Mus. No. 11.4.23.2); *D. stuhlmanni*, Burumba, Ankole, Uganda, Aug., 1903 (Brit. Mus. No. 4.2.6.33).

NOTE.—I think there can be no doubt that all the above specimens refer to *P. neumanni*. Stobbe in describing this species merely stated that it differed from *Trichodectes univirgatus* Neu. in the absence of processes on the posterior margin of the temples. The only other known species found on *Dendrohyrax* which could be confused with it are *P. congoensis* (Ferris), *P. angolensis* nov. sp. and *P. baculata* (Ferris), all of which are, *inter alia*, much smaller.

Procavicola congoensis (Ferris).

Previous records.—Types from *Dendrohyrax adolfi-friederici*, Belgian Congo; also females from *D. validus*, Mt. Kilimanjaro, but these were probably *P. baculata* (Ferris) (Ferris, 1930).

Additional records.—*D. adolfi-friederici*, Mt. Muhavura, south-west of Kigizi, Uganda, 9th Sept., 1929 (Brit. Mus. No. 30.8.1.54); *D. stuhlmanni*. Burumba, Ankole, Uganda, Aug., 1903 (Brit. Mus. No. 4.2.6.33).

NOTES.—The basal plate of the male genitalia varies in length both in this species and *P. baculata*. In Ferris' figure of the type of *P. congoensis* it is shown extending to the apex of the second abdominal segment; in the specimens from *D. adolfi-friederici* from Uganda it extends to the apex of the first segment, and in the specimens from *D. stuhlmanni* it extends to the apex of the metathorax. This species is closely related to the three following, and I have been unable to find specific characters for separating the females and those of *P. angolensis* nov. sp., except that the lobe on the inner face of the gonopophysis appears to be larger in *P. congoensis*.

Procavicola angolensis nov. sp.

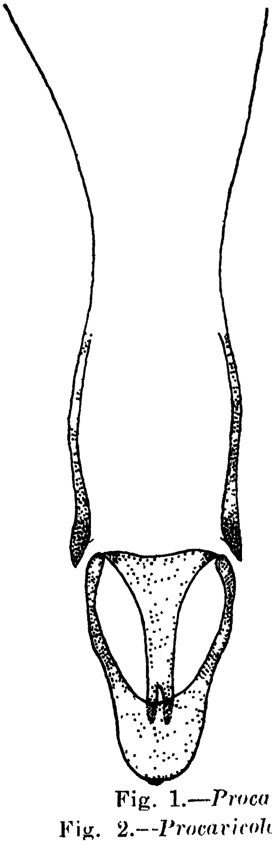
(Figs. 1 and 2).

Males and females taken off *Dendrohyrax bocagei*, Benguella, Amboim District, Angola, 27th April, 1934 (coll. K. Jordan), and *D. angolensis*, Congulu, Amboim District Angola (coll. K. Jordan). The holotype, a male, and allotype from *D. bocagei* will be deposited in the British Museum collection.

NOTES.—This species is very closely related to *P. congoensis*, of which Ferris (1930) has given excellent figures, also to *P. jordani* nov. sp., and *P. baculata* (Ferris). The male can be distinguished from both *P. congoensis* and *P. baculata* by the presence of two median transverse plates instead of only one on tergites IV to VI, and also by the male genitalia. The endomeres are fused except at their extreme apices where they are bilobed, and their latero-anterior angles are sharply pointed, whereas in both *P. congoensis* and *P. baculata* they are separated and rounded at their latero-anterior angles. The pseudopenis also differs in being more widely rounded at its apex. The basal plate is long, extending to the first abdominal segment. Preputial sac with numerous minute spines. The female, as stated above, very closely resembles that of *P. congoensis*. Both these can, however, be distinguished from the females of *P. jordani* and *P. baculata* in having two separate plates on the apical tergite, whereas in *P. jordani* and *P. baculata* these plates are united in front (compare Figs. 2, 4 and 5).

Male: Length 1·13 mm., head 0·29 × 0·26.

Female: Length 1·17 mm., head 0·29 × 0·26.



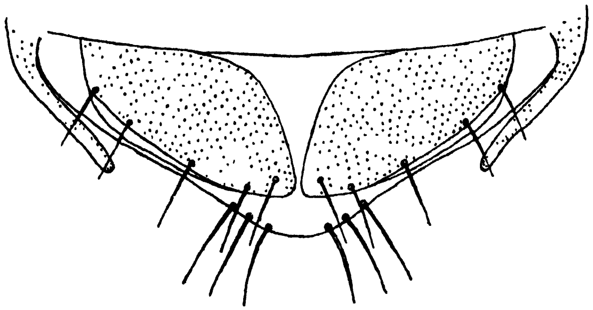


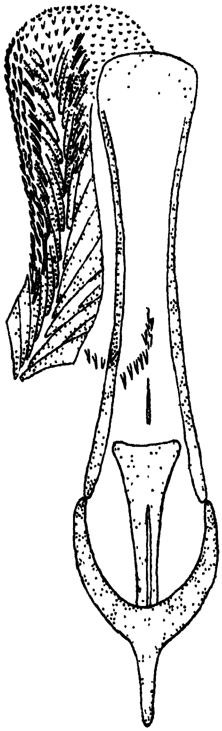
Fig. 1.—*Procavicola angolensis* nov. sp., male genitalia.
Fig. 2.—*Procavicola angolensis* nov. sp., apical tergite of female.

Procavicola jordani nov. sp.
(Figs. 3 and 4).

Males and females taken off *Dendrohyrax angolensis*, Congulu, Amboim District, Angola (coll. K. Jordan).

The holotype, a male, and allotype will be deposited in the British Museum collection.

NOTES.—This species is closely related to the two foregoing species, and also to *P. baculata*. The distinctive features in the male appear to be the presence of a narrow transverse sclerite between each of the hind coxae and the sclerotic bar on sternite i, and in the male genitalia, and in the female the apical tergite and apical sternites.



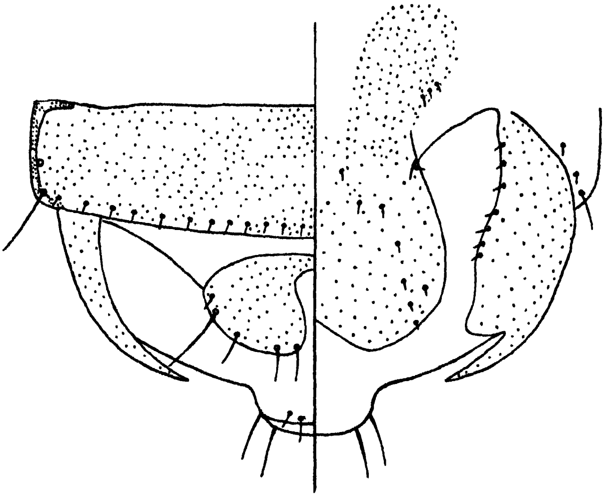


Fig. 3.—*Procavicola jordani* nov. sp., male genitalia.

Fig. 4.—*Procavicola jordani* nov. sp., apical tergites and sternites of female.

Male genitalia (Fig. 3)—The endomeres resemble *P. angolensis* except that they are situated further forward and have the latero-anterior angles rounded; above them is a minute elongated sclerite which is absent in the other species. The pseudopenis resembles that of *P. baculata* in being narrow at the apex. The basal plate is short and narrow, extending to the fifth segment. The most conspicuous feature, however, is the presence of numerous largish teeth on the preputial sac.

In the female the plates on the apical tergite are united in front as in *P. baculata*, but they are much smaller than in that species, and the apical sternites are very distinct (see Fig. 4).

Male: Length 1·15-1·2 mm., head 0·31 × 0·27.

Female: Length 1·26 mm., head 0·33 × 0·29.

Procavicola baculata (Ferris).

(Fig. 5).

Previous record.—From *Dendrohyrax validus*, Mt. Kilimanjaro (Ferris, 1930).

Additional records.—*Dendrohyrax validus*, Kilimanjaro, 30th October, 1884 (Brit. Mus. No. 85.1.17.8); and *D. neumanni*, Tambatu, Zanzibar (Brit. Mus. No. 13.10.28.5).

NOTES.—In the males from *D. validus* the basal plate of the genitalia extends into the metathorax, whereas in the males from *D. neumanni* the basal plate is slightly wider and also shorter, extending only to the third abdominal segment. No importance can be attached to this difference, however, in view of the fact that the basal plate also varies in length in males of *P. congoensis*. As stated above, this species is closely related to the three foregoing species.

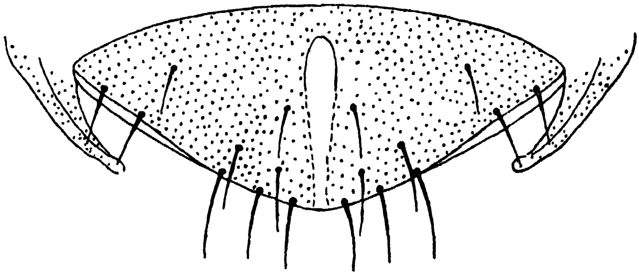


Fig. 5.—*Procavicola baculata* (Ferris), apical tergite of female.

Genus DASYONYX Bedford.

In 1932 the writer included seven species in this genus. Two more are described below, bringing the total number of described species to nine.

Dasyonyx validus Bedford.

Previous records.—Described as *Trichodectes lindfieldi* from *Dendrohyrax adolfi-friederici*, Belgian Congo, and from *D. validus*, Mt. Kilimanjaro (Ferris, 1930).

Additional records.—Males from *Dendrohyrax arborea*, Port Alfred, Cape Province, 1933 (coll. R. F. Lawrence), and males and females from *Dendrohyrax scheelei*, Ukeke District, Tanganyika Territory, 8th December, 1910 (Brit. Mus. No. 11.4.23.2.).

Dasyonyx dendrohyracis (Ferris).

Previous record.—From *Dendrohyrax validus*, Mt. Kilimanjaro (Ferris, 1930).

Additional record.—Males have been taken off the same host species, Kilimanjaro, 30th October, 1884 (Brit. Mus. No. 85.1.17.8).

Dasyonyx windhuki nov. sp.

(Figs. 6 and 7).

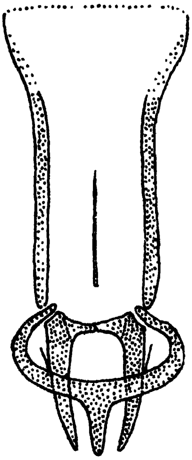
Males and females from *Procavia windhuki*, Naukluft, 1,300-1,500 m., South-West Africa, 10th December, 1933 (coll. K. Jordan), and from *Procavia* sp., Otjosongomba, Waterberg, South-West Africa (coll. K. Jordan).

Holotype, a male, and allotype from *P. windhuki* will be deposited in the British Museum collection.

This species is closely related to *D. ovalis* Bedford, described from specimens taken off *Procavia coombsi*, Weltevreden, Parys, Orange Free State. Both males and females of *D. ovalis* possess a small process on the posterior margin of each temple; these being absent in *D. windhuki*. The males can also be distinguished by the male genitalia; in *D. windhuki* they are smaller, and the arms of the pseudo-penis are shorter.

Male: Length 1·1 to 1·17 mm., head 0·22 × 0·29.

Female: Length 1·26 mm., head 0·26 × 0·33.



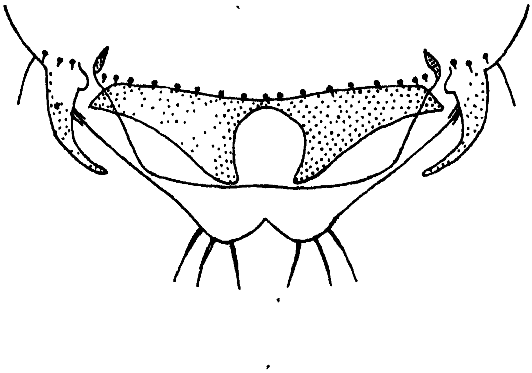


Fig. 6.—*Dasyonyx windhuki* nov. sp., male genitalia.

Fig. 7.—*Dasyonyx windhuki* nov. sp., apical sternite of female.

Dasyonyx nairobiensis nov. sp.

(Figs. 8 and 9).

Males and females from *Procavia mackinderi zelotes*, Nyong, Nr. Nairobi, Kenya Colony. *Holotype* a male.

This species is very closely related to *D. transvaalsensis* Bedford recorded from *Procavia coombsi* and *Heterohyrax granti* in the Transvaal.

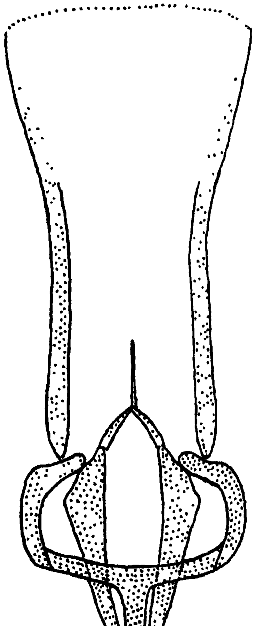
The female can be distinguished by the shape of the two transverse sclerites on sternite viii (Fig. 7), and the two triangular sclerites on the apical tergite are slightly larger.

The males can be distinguished by the genitalia and the presence of two transverse sclerites on tergites iii-vii, whereas in *D. transvaalensis* there is only one transverse sclerite on tergite vii; these sclerites also differ slightly in shape in these two species.

The genitalia are larger than those of *D. transvaalensis* and the endomeres are of a different shape and united in front by two small sclerites; immediately above these is a small longitudinal sclerite which is likewise absent in the genitalia of *D. transvaalensis*. Preputial sac with numerous minute spines.

Male: Length 1·4 mm., head 0·28-0·31 × 0·35-0·36.

Female: Length 1·59-1·62 mm., head 0·29-0·31 × 0·38-0·4.



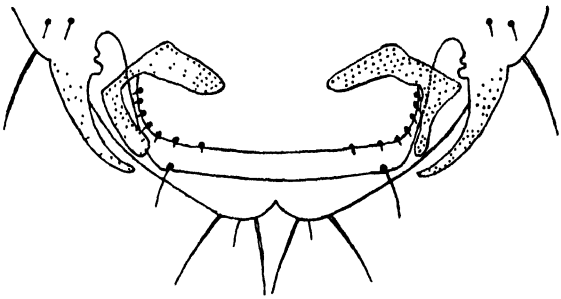


Fig. 8.—*Dasyonyx nairobiensis* nov. sp., male genitalia.

Fig. 9.—*Dasyonyx nairobiensis* nov. sp., apical sternite of female.

Genus Procaviphilus Bedford.

Procaviphilus granulatus (Ferris).

Previous records.—Females from *Dendrohyrax adolfi-friederici*, Belgian Congo (Ferris, 1930), and *D. arborea*, Port St. Johns, Cape Province (Bedford, 1932).

Additional records.—Females from *Dendrohyrax crawshayi* Thos. Solai, Mt. Kenya, Kenya Colony (Brit. Mus. No. 11.4.7.161); *D. scheelei* Matsch, Ukeke District, Tanganyika Territory (Brit. Mus. No. 11.4.23.2), and *D. stuhlmanni* Matsch. Burumba, Ankole, Uganda (Brit. Mus. No. 4.2.6.33).

Procavicola serraticus (Hill).

Previous records.—From *Procavia sp.*, Mtabamblope, Natal (L. Hill, 1922); *Procavia coombsi* Rbts., Rooikrans, Transvaal (Bedford, 1928); *Procavia natalensis* Rbts., Knysna, Cape Province, and *Procavia* sp., Lamberts Bay, Cape Province (Bedford, 1932).

Additional record.—One female from *Procavia windhuki*, Naukluft, 1,300-1,500 m., South-West Africa, 10th December. 1933 (coll. K. Jordan).

Genus BOVICOLA Ewing.

Bovicola caprae (Gurlt).

Trichodectes climax Nitzsch, *Germar's Magazine Ent.* III, p. 296 (1818), no description.

Trichodectes caprae Gurlt, *Mag. f. ges. Tierheilk.* IX, p. 3, pl. 1. f. 2 (1843).

Trichodectes climax Giebel, *Insecta Epizoa*, p. 58 (1874); Piaget, *Les Pédiculines*, p. 391, pl. 2, f. 1 (1880).

Trichodectes climax var. truncata Piaget, *ibid.*, p. 393 (1880).

In the British Museum collection there are specimens taken off goats as follows: Ilesha, S. Nigeria; Hay, New South Wales; females and males (obviously stragglers) from *Coelogenys paca* (rodent), Georgetown, British Guiana (coll. J. Rodney); Malta (coll. A. Critien), and one female, Artic Region. Also the following in the Piaget collection: Three females and one male from "une chèvre"; males, females and immature forms (types of *T. climax var. truncata*) from "une chèvre de Java"; six females, one male and one immature specimen from *Capra hircus*, and four females and one male off *Capra hircus var. indica*.

Bovicola major (Piaget).

Trichodectes climax var. major Piaget, *Les Pédiculines*, Suppl., pp. 86-87, pl. 9, f. 5 (1885).

Trichodectes painei Kellogg and Nakayama, *Psyche*, xxi, p. 90, f. 1 (1914).

In the Piaget collection (now in the British Museum) there are four females and two males (mounted on two slides) from *Capra angoriensis* labelled *Trichodectes climax*. These are most probably the types of the *var. major* as there are no specimens labelled *var. major* in the collection, and they are from the same host. As they prove to be the same as *B. painei* described by Kellogg and Nakayama, *B. painei* therefore becomes a synonym of *major*.

Harrison (1916) sank *major* as a synonym of *B. limbatus* (Gervais), discussed under *B. crassipes* (Rudow), probably because it was recorded from *Capra angoriensis*, and he was under the impression that Angora goats only harboured one species, but there are two species parasitic on these animals. Moreover, Piaget's figure of the female of *major* agrees with his specimens labelled *T. climax* from *C. angoriensis*, but not with the second species, *B. crassipes*, which is very distinct.

There are also specimens in the British Museum collection taken off a goat, Imboden, Ark. (coll. B. C. Marshall); goat, Ilminster and females without data.

This species is normally parasitic on Angora goats, but I have also taken it off Boer goats along with *B. caprae* Gurlt.

Bovicola crassipes (Rudow).

Trichodectus crassipes Rudow, *Zeit. f. ges. Natur.*, XXVII, pp. 111-112, pl. 7, f. 1 (1866).

Trichodectes penicillatus Piaget, *Les Pédiculines*, pp. 406-407, pl. 32, f. 10 (1880).

Trichodectes pilosus Piaget, *ibid*, pp. 395-396, pl. 32, f. 4 (1880), *nec.* Giebel (1874).

Trichodectes hermsi Kellogg and Nakayama, *Psyche*, XXII, p. 34 (1915).

Gervais (*Histoire Naturelle des Insectes Aptères*, 1844, III, pp. 313-314, pl. 48, f. 3, 4) described and figured two species found on Angora goats, namely, *T. climax* and *T. limbatus*. The description and figure of the former are very inaccurate, and do not agree with any of the known species found on goats. For instance, there are no transverse bands on the dorsum of the abdomen in the female, the first segment of the male antenna is much too large, and the trabecula-like processes are very different to the other species. It should therefore be discarded. It is certainly not the same as *T. climax*, recorded without description by Nitzsch in 1818, and also by Giebel (1874) and Piaget (1880). These specimens were all recorded from *Capra hircus* and are *B. caprae* (Linn.). Gervais' *T. limbatus* should likewise be discarded as it is impossible to be certain whether it is the same as *B. major* or *B. crassipes*. Had it been possible to identify *T. climax Gervais* it would have been possible to identify *T. limbatus*.. Gervais states that the body of *T. climax* is larger than that of *T. limbatus*, indicating that the former is the same as *T. crassipes*. Even if it were possible to prove this, the name *T. climax* Gervais could not be used instead of *T. crassipes* as the same name was used earlier by Nitzsch for a different insect.

Rudow's figure of *T. crassipes* is much better, and there can be no doubt that it is the same species as was described later by Piaget as *T. penicillatus* and by Kellogg and Nakayama as *T. hermsi*.

The female and male described and figured by Piaget as *T. pilosus* from a horse likewise proves to be this species. In addition to these there are five females (mounted on two slides) in the Piaget collection (now in the British Museum) labelled *T. pilosus* which prove to be *B. equi*. The types of *B. pilosus* (Piaget) must either have been stragglers or the specimens were incorrectly labelled. The same applies to *B. penicillatus* (Piaget), which is also a synonym of *B. crassipes*, and was described from a female and male (now in the British Museum) reported to have been taken off *Petrogale* (=*Macropus*) *penicillatus*. There are also specimens in the British Museum collection taken off a goat, Imboden, Ark. (coll. B. C. Marshall).

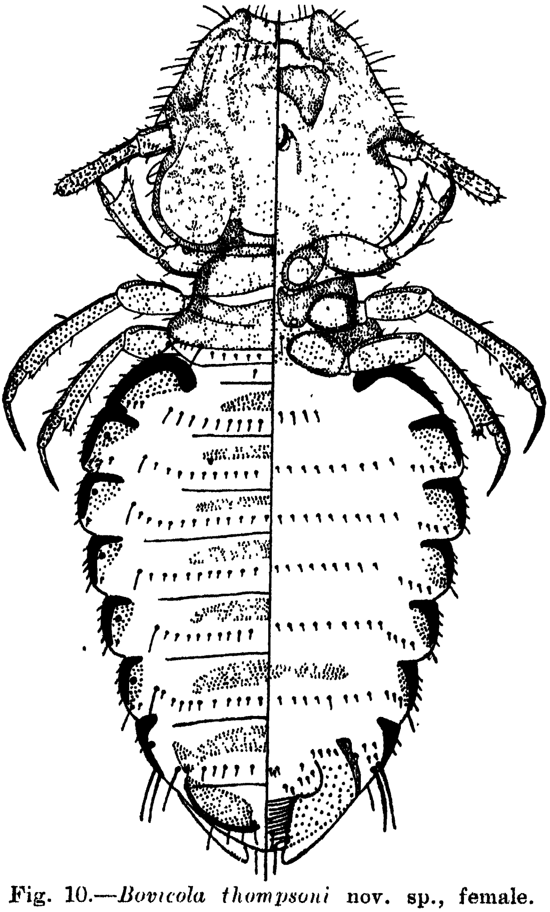


Fig. 10.—*Bovicola thompsoni* nov. sp., female.

Bovicola thompsoni nov. sp.

(Figure 10).

Several females and immature specimens were kindly forwarded by Mr. Gordon B. Thompson of the British Museum taken off *Nemorrhaedus* (=*Capricornis*) *sumatraensis* Bechstein, Barisan, Bukit, Sumatra, July, 1925. The holytype will be deposited in the British Museum collection.

Female.—Total length 2·22 mm., head 0·61 × 0·7 mm. Forehead with the anterior margin concave; temples brown with dark brown spots which are invisible in mounted specimens, and with small processes on the posterior margins. Abdomen pale with brown plates; tergites i-vii each with a median transverse plate and a single row of short setae; on the apical tergite there is a plate with four setae on each side; sternites also with a row of short setae, but transverse plates are absent except on sternite vi; paratregal plates well developed.

B. thompsoni, which is one of the largest species of *Bovicola* known, closely resemble *B. hilli* Bedford, *B. martinaglia* nov. sp. and *B. adenota* nov. sp., but the presence of processes on the temples at once distinguishes it from all other known species of *Bovicola*. Similar processes are anly known to be present in certain species ot Trichodectidae parasitic on Procaviidae.

Bovicola hilli Bedford.

Previous record.—From *Kobus ellipsiprymnus* (waterbuck), Umfolozi, Zululand (Bedford, 1934).

Additional record.—Specimens have been received from Mr. G. H. E. Hopkins taken off *Kobus defassus*, Kaiso, Uganda

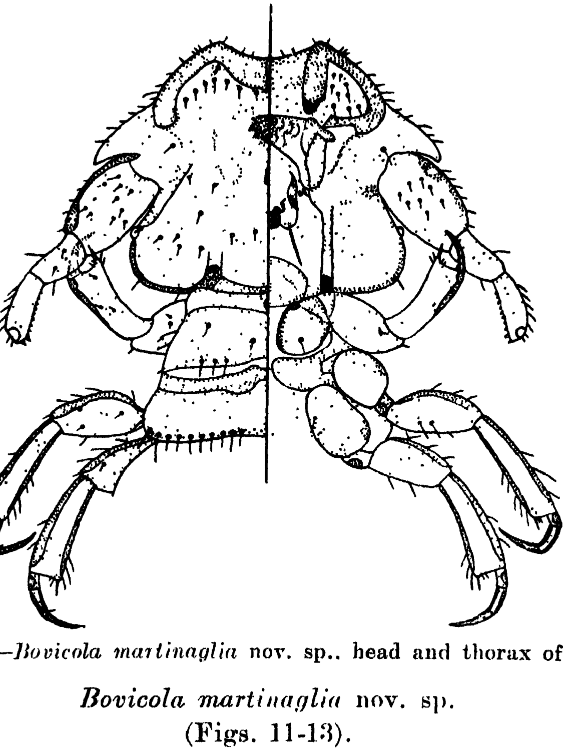


Fig. 11.—*Bovicola martinaglia* nov. sp., head and thorax of male.

Bovicola martinaglia nov. sp.

(Figs. 11-13).

Males and females were sent by Dr. G. Martinaglia taken off *Onotragus leche* in the Zoological Gardens, Johannesburg. *Holotype* a male.

Male.—Total length 1·8 mm.: head 0·42 × 0·43 mm. Forehead with the anterior margin concave; the longitudinal sclerites on the venter terminating in a spinose process in front of the base of each mandible. Antennae with the first joint enlarged; the second joint the shortest, the third with a row of minute spines on the inner margin.

Mid tibiae very slightly longer and narrower than the hind tibiae. Beneath the apex of each hind coxa there is a small transverse sclerite.

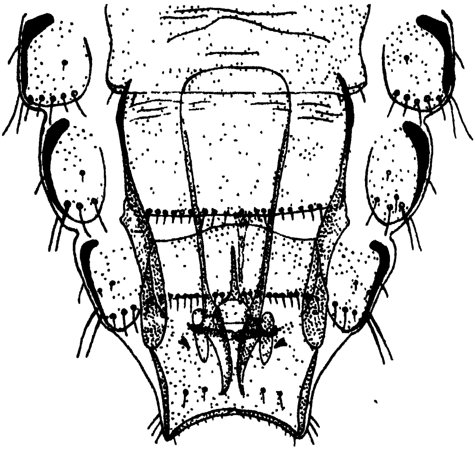


Fig. 12.—*Bovicola martinaglia* nov. sp., apical sternite of male.

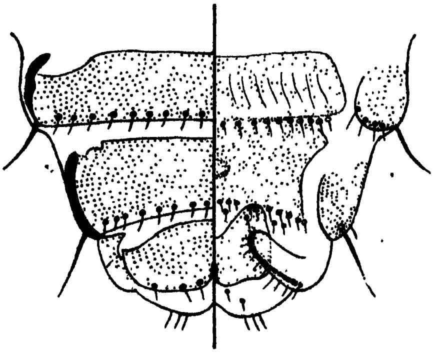


Fig. 13.—*Bovicola martinaglia* nov. sp., apical tergites and sternites of female.

Abdomen with the laterial margins crenelated, broadest at the third and fourth segments; apical segment broad at the apex with the posterior margin concave. Tergites i-vii brown with a single row of short setae, and tergites ii-iv each with lateral intersegmental furrows. Sternites i-vii brown, except for a narrow space between the median bands and paratergal plates; each with a single row of short setae. Paratergal plates well developed. Spiracles large. Genitalia as shown in Fig. 12.

Female.—Total length 1·64 mm.; head 0·38 × 0·43 mm. Differs from the male as follows: The first two antennal segments are small and subsequal, the third slightly longer than either the first or second. The median bands on the tergites and sternites are narrower, and the apical abdominal segment (shown in Fig. 13) is of a different shape.

This species is closely related to *B. thompsoni* Bedford, *B. hilli* Bedford, *B. puncta* (Piaget) and *B. adenote* nov. sp. The male can be distinguished by the apical abdominal segment and genitalia, and the female by the shape of the sclerite on the apical sternite and the gonopophyses, which are narrower and have shorter and fewer setae on their inner margins.

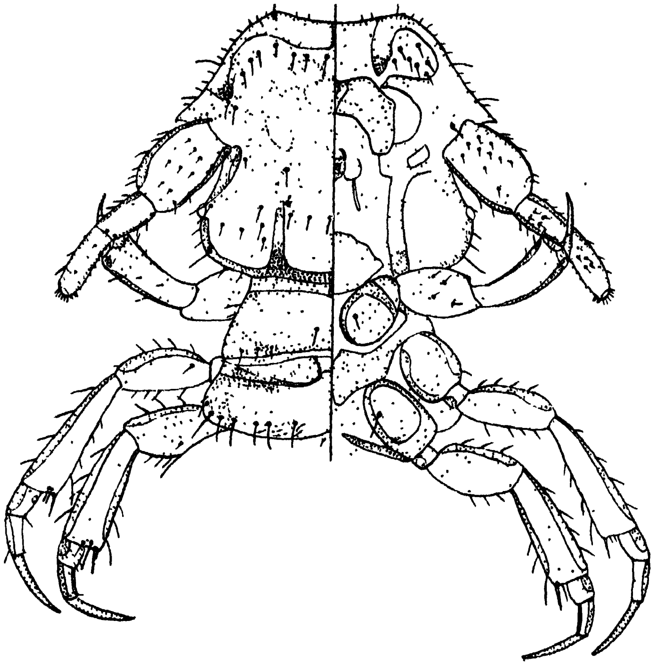


Fig. 14.

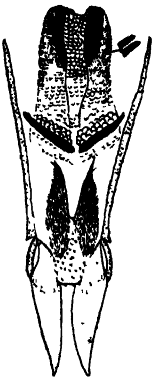


Fig. 15.

Fig. 14.—*Bovicola adenota* nov. sp., head and thorax of male.
Fig. 15.—*Bovicola adenota* nov. sp. male genitalia.

Bovicola adenota nov. sp.

(Figs. 14-17).

Males and females were kindly sent by Mr. G. H. E. Hopkins taken off *Adenota kob*, Kazinga, Uganda. *Holotype* a male.

Male.—Total length 1·85-1·96 mm.; head 0·4-0·42 × 0 42 mm. Forehead with the anterior margin slightly concave, the longitudinal sclerites on the venter terminating in a spinose process in front of the base of each mandible. Antennae with the first joint enlarged, the second joint the shortest, the third with a row of minute spines on the inner margin.

Mid tibiae very slightly longer and narrower than the hind tibiae. Beneath the apex of each hind coxa there is an elongated narrow sclerite. Abdomen elongated, with crenelated lateral margins, broadest at the third segment; apical segment broader in front than behind, with the posterior margin rounded. Tergites and sternites brown with a single row of setae, the former with lateral

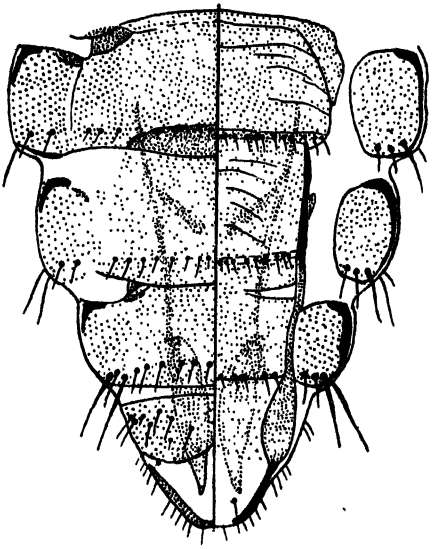


Fig. 16.—*Bovicola adenota* nov. sp., apical tergites and sternites of male.

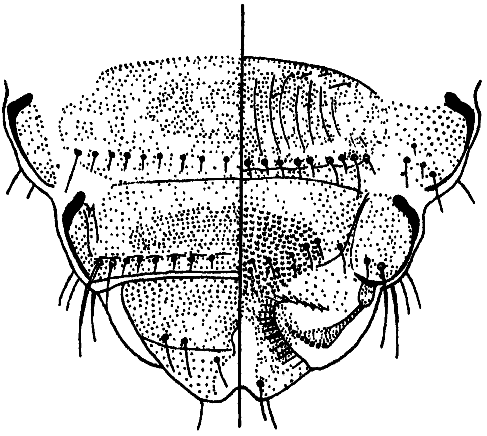


Fig. 17.—*Bovicola adenota* nov. sp., apical tergites and sternites of female.

inter-segmental furrows, these being more developed on tergites ii-v. Parateigal plates well developed. Male genitalia with the parameres short and curved; endomeres larger and elongated; basal plate with two elongated sclerites near the middle; preputial sac with numerous small spines.

Female.—Total length 1·83-1·87 mm., head 0·43×0·47 mm. Differs from the male as follows: The first two antennal segments are short and subequal, the third very slightly longer than either the first or second; the sclerites beneath the hind coxae are much shorter, and the bands on the abdomen are smaller.

This species is closely related to the three foregoing species from which it can be distinguished by the shape of the forehead, the anterior of which is less concave. Other distinguishing characters are to be found in the male genitalia, and the apical abdominal segments of the female.

Genus DAMALINIA Mjoberg.

Damalinia forficula (Piaget).

Trichodectes forficula Piaget, *Les Pédiculines*, pp. 400-402, pl. 32, f. 7 (1880).

Previous record.—From *Cervus porcinus*, Zoological Gardens, Rotterdam.

Additional record.—Specimens have been received from Dr. G. Martinaglia who took them off *Cervus axis* in the Zoological Gardens, Johannesburg. The host came from Calcutta, India.

Damalinia hopkinsi nov. sp.

(Figs. 18 and 19).

Two males received from Mr. G. H. E. Hopkins taken off an eland (*Taurotragus oryx pattersonianus*) at Gayaza, Uganda, 25th July, 1934.

Male.—Total length 2·15-2·2 mm.; head 0·28×0·26 mm. Forehead with a V-shaped notch in front. Antennae with the first segment long and broad, the second and third subequal. Mid tibiae slightly longer and narrower than the hind tibiae. Abdomen elongated, with crenellated lateral margins, widest at the third segment, and ending posteriorly in two long chitinous lobes. Tergites i-iii each with a single brown median sclerite, the first two more chitinous on the median portion of their posterior margins, where they project backwards on each side of the middle, forming two short spines. Tergites iv-vii each with two median sclerites. Paratergal plates well developed. Prothoracic and abdominal spiracles large, present on abdominal segments ii-vii. Genitalia with the basal plate long and narrow; parameres very small; preputial sac with numerous minute teeth.

This species is closest to *D. forficula* (Piaget) and *D. theileri* Bedford from which the male can be distinguished by the posterior margins of tergites i-ii, the genitalia, and the apical abdominal segment. *D. theileri* is a much larger species.

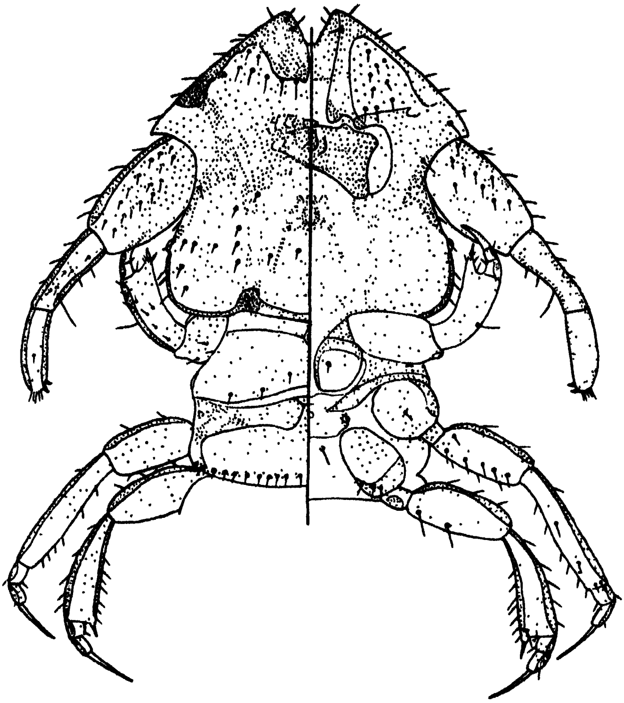


Fig. 18.—*Damalinia hopkinsi* nov. sp., head and thorax of male.

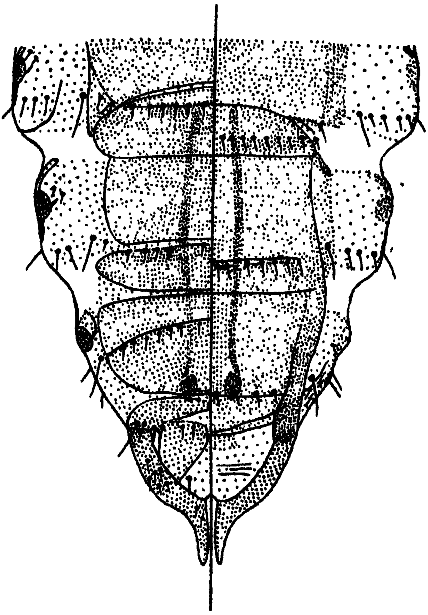


Fig. 19.—***Damalinia hopkinsi*** **nov. sp., apical tergites and sternites of male.**

Genus TRICHODECTES Nitzsch.

Trichodectes ovalis Bedford.

Trichodectes ovalis Bedford, *Repts. Dir. Vet. Educ. & Res., Un. S. Afr.*, XIII-XIV, p. 841, pl. 1, f. 1, 3; pl. 6, f. 13 (1928).

Previous records.—From *Poecilogale albinucha*, Onderstepoort (Bedford, 1928) and *Ictonyx striatus*, South-West Africa and Natal (Bedford, 1929).

Additional record.—Specimens received from Mr. G. H. E. Hopkins taken off *Ictonyx striatus*, Mt. Sabinia, Kigezi, Uganda, 24th November, 1934.

In view of the fact that *T. ovalis* has been found three times on *Ictonyx striatus* and a new species on *Poecilogale doggeti*, I feel convinced that the host from which the type specimens of *ovalis* were collected was misidentified by the writer.

Trichodectes ugandensis nov. sp.

(Figs. 20 and 21).

Two females and one male kindly sent by Mr. G. H. E. Hopkins taken off *Poecilogale doggetti* Thos., Mt. Sabinia, Kigezi, Uganda, 23rd November, 1934. *Holotype* the male.

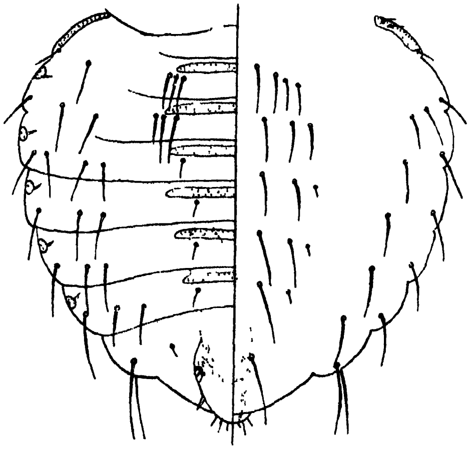


Fig. 20.—*Trichodectes ugandensis nov. sp.*, abdomen of male.

This species is very closely related to *T. ovalis* Bedford, from which it mainly differs in the chaetotaxy of the abdomen, the shape of the sclerites on the apical tergites in the female, and male genitalia.

Male.—Total length 1·03 mm., head 0·31 x 0·36 mm. Forehead slightly rounded in front with a shallow median notch; the lateral sclerites project backwards for a short distance in front where they are separated by a clear space, and terminated in a short spinose process in front of the antennae. In front of each eye there is a dark spot. Temples rounded, with a narrow marginal sclerite. Occipital sclerites dark at their bases where they are connected by a narrow sclerite. Antennae with the segments sub-sequal in length, the first segment with four short setae on the dorsum near the apex.

Prothorax broader than long, with a single seta on each side of the meson, and two short ones on each lateral margin. Pterothorax broader than prothorax, with three setae at each latero-anterior margin.

Legs short, the coxae widely separated, especially the mid and hind coxae; between the fore and mid coxae there is a narrow sclerite which broadens posteriorly between the mid coxae.

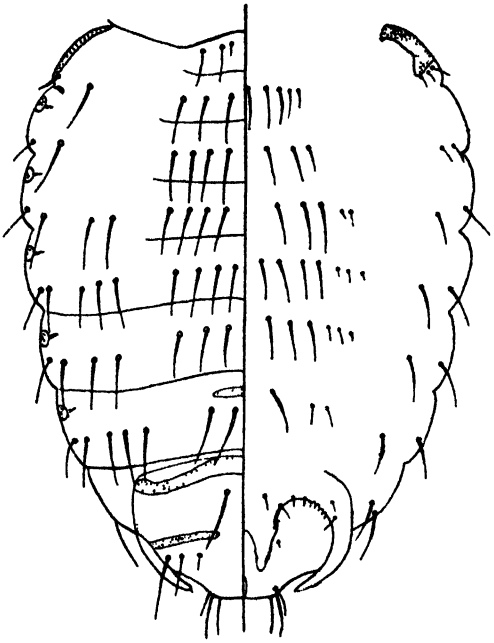


Fig. 21.—*Trichodectes ugandensis nov. sp.*, abdomen of female.

Abdomen oval, slightly wider at the third segment than long. Tergites i-vi each with a narrow, median, transverse sclerite; beneath the lateral margins of the first two sclerites there are three well-developed setae, and beneath the remaining four sclerites there is one short seta on each side. Sternite i with a median transverse row of eight setae and three on each side; sternites ii-v with six median setae, the lateral ones short except on the third, and two on each side; sternite vi with four median setae and two on each side, and sternite vii with two median setae. Paratergal plates absent, except on the first segment. Spiracles large, present on the prothorax and abdominal segments ii-vi.

Female.—Total length 1·36 mm.; head 0·35 × 0·42 mm. Head as in the male. Antennae slightly narrow, the apical segment longer than either the first or second segments. Pro- and ptero-thorax as in the male, except the latter has one or two setae on each side of the meson.

Abdomen elliptical, without sclerites, except for an inconspicuous median one on the sixth tergite, a narrow transverse sclerite in the middle of the seventh, and a short transverse one on each side of the eighth tergite. Tergites i-v each with a median row of six to eight setae and from one to five on each side; tergite vi with four median setae and five on each side, and tergite vii with two median setae. Sternite i with a median row of ten setae; sternite ii with six; sternite iii with six long and two short on each side; sternite iv with eight long and three short on each side; sternite v with six long and three short on each side; sternite vi with two long and two short on each side, and sternite vii with two short setae.

Both the males and females of this new species can be distinguished from those of *T. oralis* by the setae on the abdomen being less numerous both on the dorsum and venter. In the female of *T. oralis* the transverse sclerite on tergite vii is stright, and on tergite viii there is a single median transverse sclerite.

In addition to both *T. oralis* and *T. ugandensis* the following species also have the lateral sclerites on the forehead terminating posteriorly in spinose processes: *T. canis* (Degeer), *T. potus* Werneck, *T. melis* (Fabr.), *T. rosseleri* Stobbe, *T. octomaculatus* Paine, *T. barbarae* Neu., *T. galictidis* Werneck, *T. mustelae* (Schrank) and *T. pallidus* Piaget (synonym *T. nasuatis* Osborn). Similar processes are also present on the venter of the head at the bases of the trabecula-like processes in the majority of the above species.

Trichodectes galictidis Werneck.

Trichodectes mephitidis Neumann, *Archiv. Parasit*, XV, p. 618, f. 10 (1911), *nec* Packard, 1872.

Trichodectes galictidis Werneck, *Mem. Instit. Oswaldo Cruz*, XXVIII, i, p. 162, t.f. 1-5 (1934).

Previous records.—From *Galictis quiqui*, Chili and *Helictis emeretti* (Neumann, 1911); also from *Galictis vittata*, Brasil (Werneck, 1934).

Additional record.—Specimens kindly sent by Mr. L. H. Dunn taken off *Grisonia canaster* (Nelson), Pacora, Panama.

Genus LORISICOLA nov.

Small species. Head much broader than long; forehead short, with a very narrow, shallow notch in front; sclerites on lateral margins with a backward projecting spinose process midway between the anterior margin and trabecula-like processes. A pair of similar processes on verter of head in front of the mandibles. Pharyngeal sclerite present. Pterothorax very broad, winged at the antero-lateral margins. Abdomen with paratergal plates, those on the third segment the largest and lobed as in species of *Procavicola* found on rock rabbits. Tergites and sternites with narrow median transverse bands. Spiracles present on abdominal segments ii to vii. Genital plate of female with two backward-projecting spines on each side. Gonopophyses of female narrow, with a few short setae on inner margins. Male with large genital plate; genitalia with parameres forming a pseudo-penis.

Species found on Asiatic lemurs belonging to the sub-family Lorisinae.

Genotype.—*Trichodectes mjöbergi* Stobbe.

Trichodectes abnormis Ewing, described from a male taken off *Lemur rufus*, Madagascar, appears to be very distinct, and cannot be included in this genus.

Lorisicola mjöbergi (Stobbe).

Trichodectes mjöbergi Stobbe, *Sitz-Ber. Ges. nat. Freunde*, p. 379, f. 8 (1913).

Trichodectes brachycephalus Ewing, *Proc. Ent. Soc. Wash.*, XXXII, vii, p. 120 (1930).

Previous records.—Both sexes from *Nycticebus borneanus*, North Borneo (Stobbe), and a male off *Nycticebus coucang*, Johor Lama, Malay Peninsula (Ewing).

Additional record.—Both sexes from *Nycticebus buku*, West Coast, Sumatra (sent by Mr. G. B. Thompson).

Genus CEBIDICOLA nov.

Large species. Head slightly broader than long; forehead elongated, sub-triangular, with a deep notch in front; sclerites on lateral margins united in front where they broaden out behind the notch, with (*armatus*) or without (*subarmatus*) backward projecting processes anteriorly and posteriorly. Pharyngeal sclerite present. Abdomen with paratergal plates and narrow, median, transverse bands. Spiracles present on abdominal segments ii-vii. Gonopophyses of female broad, with numerous setae on their inner margins. Male genitalia with the parameres projecting outwards, then backwards and inwards, but not forming a pseudo-penis.

Species found on American spider monkeys.

Genotype.—*Trichodectes armatus* Neumann.

Trichodectes subarmatus Neumann must also be included in this genus.

Genus FELICOLA Ewing.

Felicola Ewing, A Manual of External Parasites, pp. 122, 192 (1929).

Suricatoecus Bedford, *Parasit*, XXIV.

Felicola cooleyi Bedford.

Previous record.—From *Suricata suricatta hamiltoni*, Pretoria District, Transvaal.

Additional record.—Specimens received from Mr. G. H. E. Hopkins taken off *Mungos mungo colonus*, Uganda.

The types are not as well developed as are the specimens from *M. mungo colonus*, which prove to be typical *Felicola*. *Suricatoecus* must, therefore, be sunk as a synonym of *Felicola*.

Felicola zeylonica nov. sp.
(Figs. 22 and 23).

Males and females received from Mr. Gordon B. Thompson taken off *Herpestes vitticollis*, Gammaduwa, Mousakande, Ceylon (coll. W. W. A. Phillips). The holotype, a male, and allotype will be deposited in the British Museum collection.

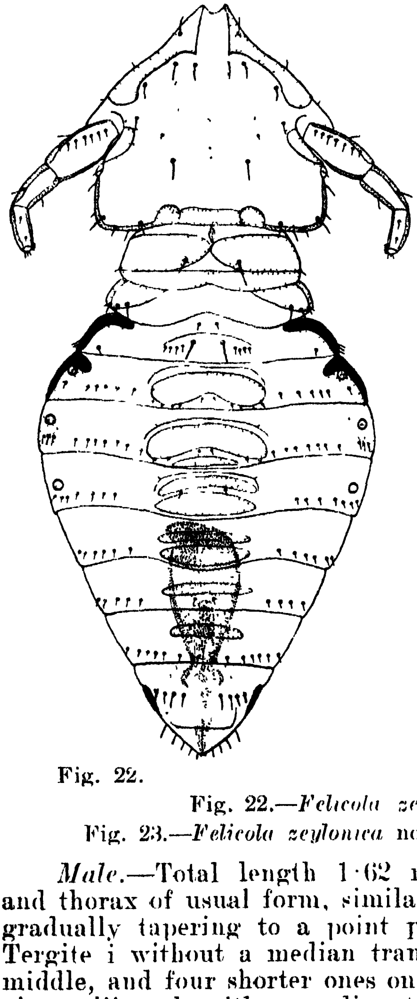


Fig. 22.

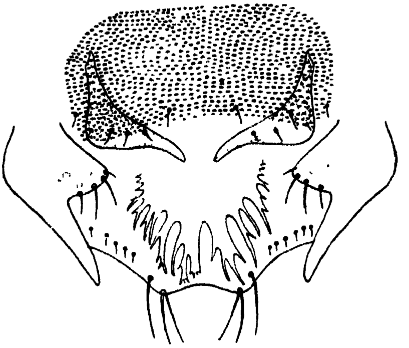


Fig. 23.

Fig. 22.—*Felicola zeylonica* nov. sp., male.
Fig. 23.—*Felicola zeylonica* nov. sp., apical sternites of female.

Male.—Total length 1·62 mm.; head 0·47 × 0·5 mm. Head and thorax of usual form, similar to *F. rammei* (Stobbe). Abdomen gradually tapering to a point posteriorly from the third segment. Tergite i without a median transverse band, with two setae in the middle, and four shorter ones on each side of them. Tergites ii and vi to viii each with a median transverse band; on tergites iii to v the bands are duplicated. Sternites with indistinct median bands; sternite viii with a small sclerite on each lateral margin. Paratergal plates present on segments i to iii. Spiracles large, present on segments ii to v. Genitalia with the parameres short, narrow and curved; endomeres elongated; preputial sac with numerous minute and narrow, elongated spines.

Female.—Total length 1·78 mm.; head 0·54 × 0·57 mm. Head and thorax as in the male. Antennae with the first segment slightly shorter than the second, which is equal to the third segment.

Abdomen oval, widest at the third segment. Tergite i with six setae in the middle. Tergites ii to vii each with a long, median transverse band and a transverse row of minute setae; tergite viii with a basal transverse band which projects backwards to a point in the middle and broadens out on each side; on the posterior margin there is a row of nine to ten setae. Sternites iii to vi each with a transverse row of short setae; apical sternites as in Fig. 23. Paratergal plates present on segments i to v.

This species is closely related to *F. rammei* (Stobbe), from which the male can be distinguished, *inter alia*, by the presence of two median bands on the third tergite, the basal band on the eighth tergite is short and does not extend to the lateral margins, the lateral plates on the eighth sternite, these being absent in *rammei*, and the parameres are much shorter. The female can be distinguished from that of *rammei* in possessing a triangular sclerite on each side of the meson above the gonopophyses.

Felicola subrostrata (Nitzsch).

Previous records.—From domestic cats in Europe, America and South Africa.

Additional record.—One female sent by Mr. G. B. Thompson taken off a wild cat, *Felis silvestris grampis* (Miller), Dundonnell, Wester Ross, Scotland.

Felicola hopkinsi nov. sp.

(Figs. 24-26).

One male and one female were received from Mr. G. H. E. Hopkins taken off *Nandinia binotata arborae*, Kampala, Uganda, 13th September, 1933. *Holotype* the male.

Male.—Total length 1·13 mm.; head 0·31 × 0·31 mm. Head similar in shape to other species found on genets. Antennae with the first segment about as long as the second and slightly broader.

Prothorax with very large spiracles, and one minute seta on each side of the meson. Pterothorax with three minute seta on the lateral angles.

Abdomen gradually tapering to a point from the third segment. Tergite i with a thick seta on each side of the meson and a small sclerite above it; tergites ii to iv each with a median transverse band, one minute seta on each side of the meson, and three or four lateral to these; tergite v with two median transverse bands, a pair of admedian setae between them, and three on each side; tergites vi and vii each with one median band, two admedian setae, and four or five on each side. Apical tergite elongated, with a longitudinal band on each side of the meson, and several setae at the apex. Sternites without bands. Paratergal plates present on segments i to iii. Spiracles absent. Genitalia with the basal plate short, parameres united, forming a loop; endomeres very long and slender.

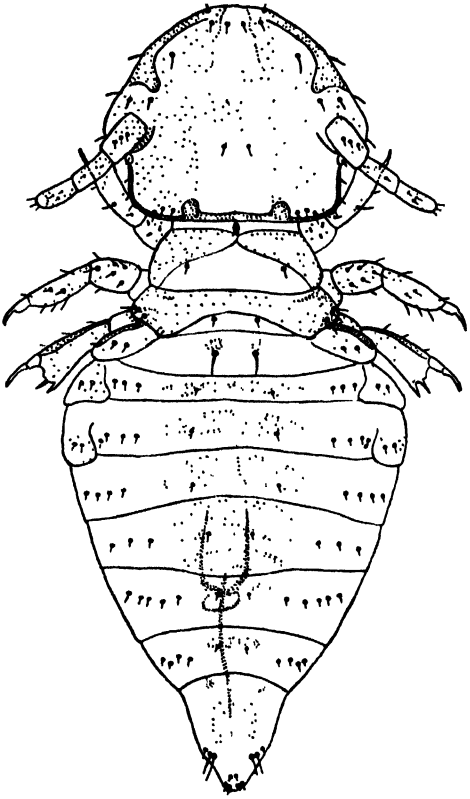


Fig. 24.—*Felicola hopkinsi* nov. sp., male.

Female.—Total length 1·38 mm.; head 0·35 × 0·36 mm. Head, antennae and thorax as in the male. Abdomen elongated oval. Tergites i to vii each with a median transverse band terminating in a narrow up-curved point, except those on the basal segments. Sternites without bands, except for a small one on the sixth sternite. Apical sternite with a bi-lobed sclerite between the gonopophyses.

F. hopkinsi resembles *F. acuticeps* (Neumann) (synonym *F. genetta* (Bedford), which is also parasitic on genets, in the shape of the head. The male of *F. hopkinsi* can be distinguished by the bands on the tergites, the apical abdominal segment being long and pointed, and the male genitalia, which are very distinct. The female can be distinguished by the plate on the apical sternite.

Specimens of *F. acuticeps* were received from Mr. G. H. E. Hopkins taken off *Genetta tigrina stuhlmanni*, Kigowa, Uganda.

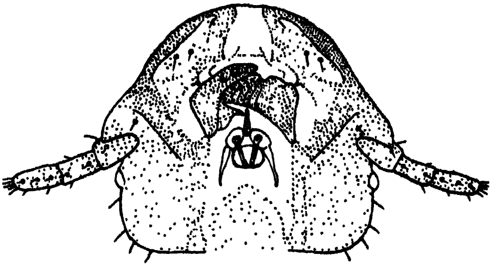


Fig. 25.—*Felicola hopkinsi* nov. sp., venter of head of female.

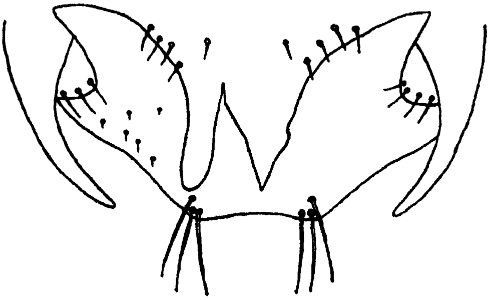


Fig. 26.—*Felicola hopkinsi* nov. sp., apical sternite of female.

HOST LIST OF THE SPECIES OF TRICHODECTIDAE FOUND ON PROCAVIIDAE

Dendrohyrax adolfi-friederici Brauer. Belgian Congo, Uganda.
Eurytrichodectes paradoxus Stobbe.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).
Procavicola congoensis (Ferris).
Dasyonyx validus Bedford.
Procaviphilus granulatus (Ferris).

Dendrohyrax arborea (A. Smith). South Coast of Cape Province.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).
Dasyonyx validus Bedford.
Procaviphilus granulatus (Ferris).

Dendrohyrax bocagei (Gray). South West Africa.
Procavicola univirgata (Neu.).
Procavicola angolensis nov. sp.

Dendrohyrax angolensis. Angola.
Procavicola univirgata (Neu.).
Procavicola angolensis nov. sp.

Dendrohyrax crawshayi Thos. Kenya Colony.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).
Procaviphilus granulatus (Ferris).

Dendrohyrax neumanni Matsch. Zanzibar.
Procavicola baculata (Ferris).

Dendrohyrax scheelei Matsch. Tanganyika Territory.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).
Dasyonyx validus Bedford.
Procaviphilus granulatus (Ferris).

Dendrohyrax stuhlmanni Matsch. Uganda.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).
Procavicola congoensis (Ferris).
Procaviphilus granulatus (Ferris).

Dendrohyrax validus True. Kilimanjaro.
Procavicola congoensis (Ferris).
Procavicola baculata (Ferris).
Dasyonyx validus Bedford.
Dasyonyx dendrohyracis (Ferris).

Dendrohyrax sp. Locality?
Eurytrichodectes paradoxus Stobbe.
Procavicola univirgata (Neu.).
Procavicola neumanni (Stobbe).

Heterohyrax brucei bakeri (Gray). Uganda.
Procavicola sp. Recorded as *P. sternata* (Bedford).
Procaviphilus ferrisi Bedford.

Heterohyrax granti Wroughton. Northern Transvaal.
Procavicola lindfieldi (Hill).
Procavicola heterohyracis Bedford.
Dasyonyx transvaalensis Bedford.
Procaviphilus sclerotis Bedford.

Heterohyrax pumila rudolfi (Thomas). Kenya Colony.
Procaviphilus ferrisi Bedford.

Heterohyrax ruddi (Wroughton). Northern Transvaal, Portuguese East Africa.
Procavicola lindfieldi (Hill).
Procavicola emarginata Bedford.
Dasyonyx oculatus (Bedford).
Procaviphilus robertsi (Bedford).

Procavia coombsi Roberts. Transvaal, Orange Free State.
Procavicola pretoriensis Bedford.
Dasyonyx ovalis Bedford.
Dasyonyx transvaalensis Bedford.
Procaviphilus serraticus (Hill).

Procavia mackinderi zelotes. Kenya Colony.
Dasyonyx nairobiensis nov. sp.

Procavia natalensis Roberts. Pigg's Peak, Swaziland; Deepdale. Natal; Grahamstown and Knysna, Cape Province.
Procavicola lindfieldi (Hill).
Procavicola natalensis Bedford.
Procaviphilus serraticus (Hill).

Procavia waterbergensis Brauer. South West Africa.
Dasyonyx waterbergensis Bedford.

Procavia windhuki. South West Africa.
Dasyonyx jordani nov. sp.
Procaviphilus serraticus (Hill).

Procavia sp. South West Africa.
Dasyonyx jordani nov. sp.

Procavia sp. Mtabamhlope, Natal.
Procavicola lindfieldi (Hill).
Procavicola sternata (Bedford).

Procavia sp. Mount Fletcher, Cape Province.
Procavicola subparva Bedford.

Procavia sp. Lambert's Bay, Cape Province.
Procavicola parva Bedford.
Procaviphilus serraticus (Hill).

Procavia syriacus (Schreber).
Dasyonyx diacanthus (Ehrenberg).

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

New Species of Linognathus and Polyplax *(Anoplura).*

By G. A. H. BEDFORD, Section of Parasitology, Onderstepoort

Linognathus peleus nov. sp

(FIGS 1-2.)

MALES and females taken off *Pelea capreolus* (Vaal Rhebok), Naauwpoort, C.P., 26th July, 1922 (coll. Austin Roberts). Holotype a male.

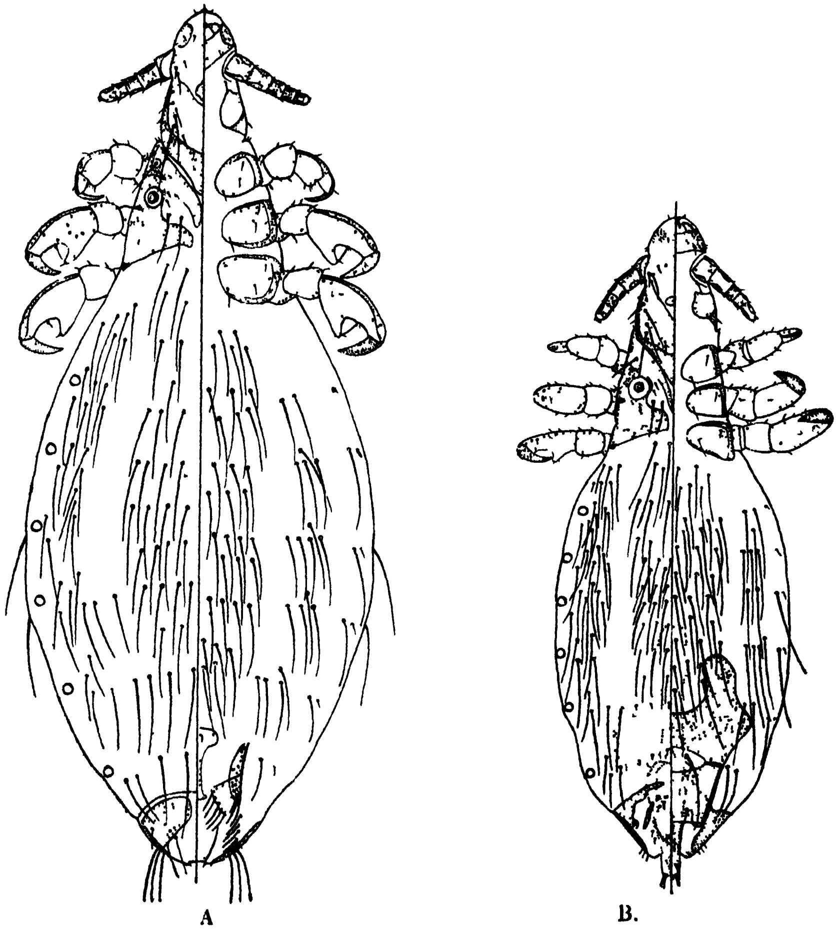


Fig. 1.—*Linognathus peleus* nov. sp., female (A) and male (B).

Female (Fig. 1A).—Length 2·1 mm. *Head* elongate, the forehead parabolic with a transverse band on the venter; antennae set well forward; hind head with the lateral margins gradually broadening from base to apex; dorsum with a distinct, irregular sclerotic pattern, the setae of medium length; pharynx with brushes, mouthparts extending beyond posterior margin of the head.

Thorax shorter than the head; sternal plate absent.

Abdomen elongate oval, the setae arranged dorsally and ventrally into median and lateral groups. *Spiracles* large with distinct markings. *Gonopophyses* (Fig. 2A) elongate with a few short setae on their inner margins; genital plate spatulate.

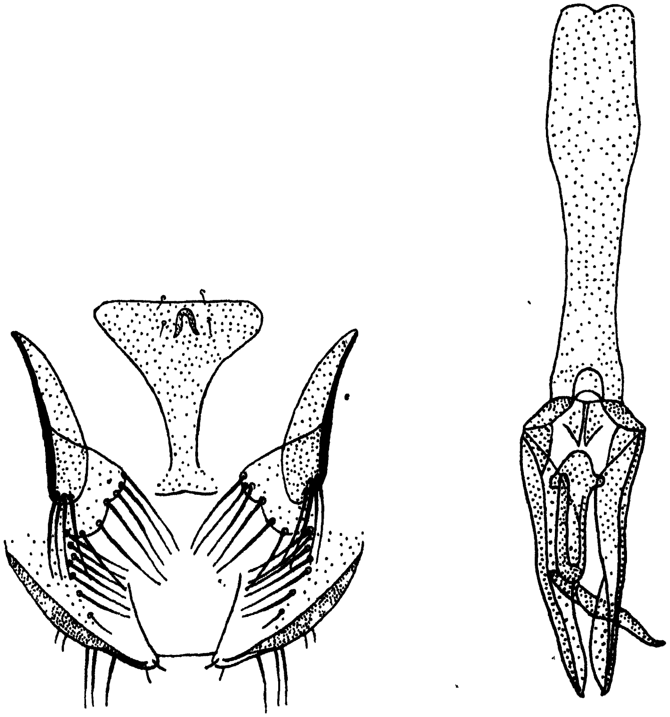


Fig. 2.—*Linognathus peleus* nov. sp., A. genital region of female; B. male genitalia.

Male (Fig. 1B).—Length 1·6 mm. Head and thorax as in the female. *Abdomen* terminating posteriorly in a pointed process. *Genitalia* (Fig. 2B) with the basal plate long and slender, likewise the parameres; endomeral piece well developed.

A very distinct species apparently belonging to the *tibialis* group. It can be distinguished by the shape of the head, the female genital region, male genitalia, and apex of the abdomen of the male.

Linognathus damaliscus nov. sp.

(Figs. 3-4.)

Males and females (including the holotype, a male, and allotype) taken off *Damaliscus albifrons* (blesbok), Zoological Gardens, Johannesburg (coll. G. Martinaglia), and females off *Damaliscus dorcas* (Bontebok), Bredasdorp, C.P. (coll. Austin Roberts)

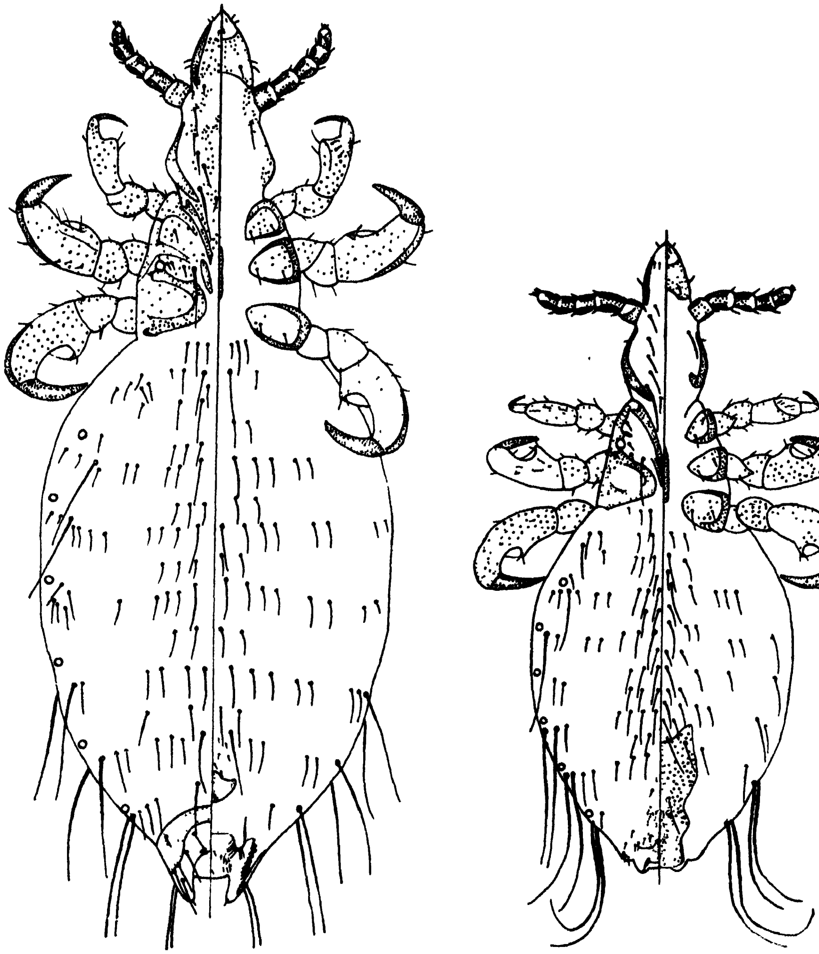

A. B.

Fig. 3.—*Linognathus damaliscus* nov. sp., female (A) and male (B).

Female (Fig. 3A).—Length 1·75 mm. *Head* elongate; the forehead acutely pointed with a transverse band on the venter; antennae elongate, set about the middle; dorsum with a distinct, irregular sclerotic pattern, the setae of medium length; hand head with lateral margins convex and sclerotic; pharynx with brushes; mouth-parts extending beyond posterior margin of head.

Thorax shorter than the head; sternal plate present, very narrow.

Abdomen elongate oval, sparcely haired, the setae which are short and slender arranged in normal pattern. *Spiracles* medium sized without markings. *Gonopophyses* (Fig. 4A) small, rounded posteriorly, with a row of setae on their inner margins; genital plate subtriangular.

Male (Fig. 3B).—Length 1·55 mm. Head and thorax as in the female. Abdomen terminating posteriorly in a short, broad lobe. Genitalia (Fig. 4B) with the basal plate long and slender; parameres each with a pointed expansion on their inner margins and pointed at their apices; endomeral piece elongate, extending almost to the base of the parameres and pointed in front. This species belongs to the *tibialis* group. The female can be distinguished by the shape of the genital plate and gonopophyses, and the male by the genitalia, especially the shape of the endomeral plate.

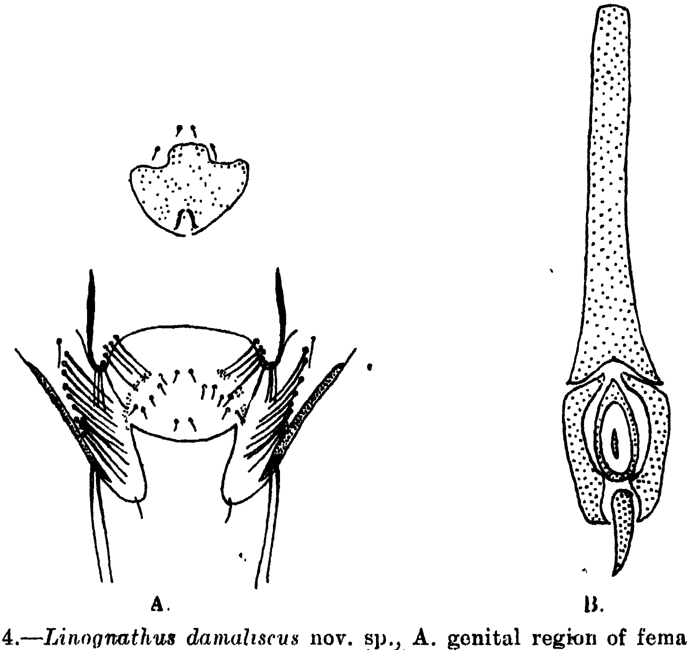


Fig. 4.—*Linognathus damaliscus* nov. sp., A. genital region of female; B. male genitalia.

Linognathus aepycerus nov. sp.

(Figs. 5-6.)

Males and females (including the holotype, a male, and allo type) taken off *Aepyceros melampus* (Impala), between Pretoria and Johannesburg; also females (mostly slightly immature) off some host species, Rustenburg District, Transvaal.

Female.—Length 1·85–2 mm. *Head* short and broad; forehead rounded; antennae situated slightly forward beyond the middle; hind head with lateral margins distinctly angulate and constricted posteriorly; pharynx with well-developed brushes; mouth-parts extending beyond posterior margin of the head.

Thorax short and broad, of ordinary form; sternal plate present, slender.

Abdomen elongate oval, similar to other species of the *breviceps* group, the setae sparse and short except for a median pair on each segment dorsally and ventrally; long marginal setae present on segments vi to viii, and a single long seta near the spiracle on the second segment. *Spiracles* small. *Gonophyses* (Fig. 6A) elongate with long setae on posterior margin; genital plate racquet-shaped.

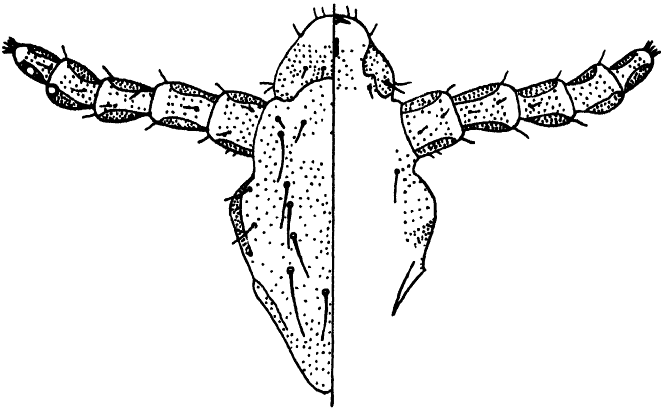


Fig. 5.—*Linognathus aepycerus* nov. sp., head of male.

Male.—Length 1·19 mm. Similar to the female in general characters, except that the abdomen is pointed posteriorly. *Genitalia* (Fig. 6B) with the basal plate long and slender; parameres slender, without notches on their inner margins; endomeral piece long and slender, pointed in front. This species belongs to the *breviceps* group. Both sexes can be distinguished from *L. angulatus* (Piaget) by the shape of the head, and the male also by the genitalia. In the male of *L. angulatus* the parameres are slightly notched at their apices. From other species belonging to the group the females can be distinguished, *inter alia*, by the shape of the genital plate, and the males by the genitalia.

Polyplax subtaterae nov. sp.

(Fig. 7.)

Males and females taken off *Tatera liodon smithii*, Kampala, Uganda (coll. G. H. E. Hopkins). *Holotype* a female.

Female.—Length 1·34 mm. *Head* slightly longer than broad; antennae set close to the anterior margin, which is almost truncate; hind head with prominent post-antennal angles and strongly constricted occipital region, lateral margin straight. Antennae with the first segment dilated.

Thorax about as long as the head, with the lateral angles roundly convex; on the dorsum there is one short seta on each side above the spiracle and a long one on the submargin. Sternal plate as in fig. 7B. *Abdomen* with the tergal and sternal plates short and very narrow, the tergal plates of the second and eighth segments each with about eight setae, and the remainder with about twenty or more setae. Sternal plate of the second segment with ten setae, those of the median segments with about seventeen setae, and of the seventh six setae; between the ends of each sternal plate on the third to seventh segments and the corresponding paratergal plates there is a single seta.

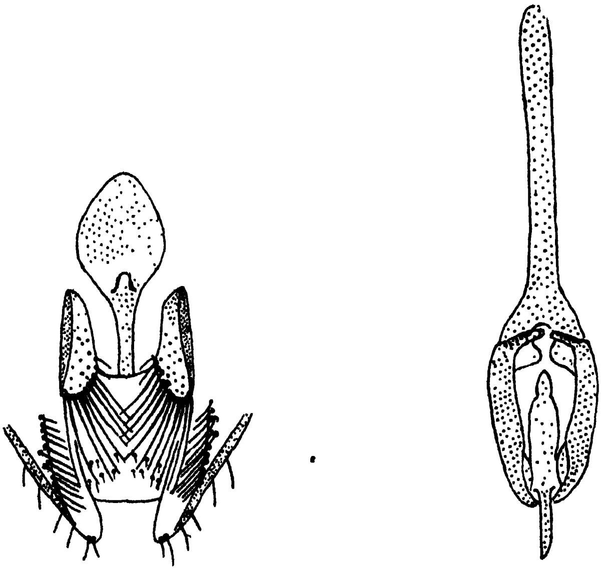


Fig. 6.—*Linognathus aepycerus* nov. sp., A. genital region of female; B. male genitalia.

Paratergal plates (fig. 7A) as follows: Those of the second segment distinctly divided longitudinally, each portion with a single seta, and the dorsal portion with a tapering process; plates of the third to sixth segments each with a small tooth at each posterior angle; those of the third and fourth segments each with a short ventral seta and a very long dorsal seta; those of the fifth and sixth segments with two very short setae; plates of the seventh and eighth segments small, especially the latter, each with two long setae.

Male.—Length 1·15 mm. *Head* about as broad as long, with the lateral margins of the hind head rounded, otherwise as in the female. Antennae with the basal segment much dilated and with the third segment strongly modified. Thorax similar to that of the female.

Abdomen as in *P. werneri* (Glink), except that the sternal plates are smaller, and there is only one seta on each of the median segments between the sternal plates and the corresponding paratergal plates; also the long setae on the third and fourth paratergal plates are much longer, being as long as the three following segments. *Genitalia* as in *P. werneri.* This species belongs to the *praecisa* group, and appears to be intermediate between *P. taterae* Ferris and *P. werneri* (Glink). From *P. taterae*, *P. praecisa* and *P. biseriata* it can be distinguished in having the short setae on the paratergal plates much shorter, and the male also by the genitalia. From *P. werneri* the male can be distinguished, apart from the characters given above, by the shape of the sternal plate.

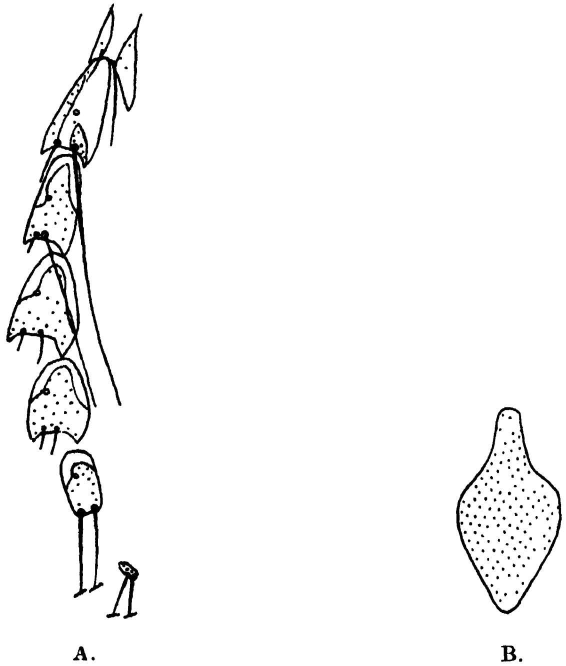

A. B.

Fig. 7.—*Polyplax subtaterae* nov. sp., A. paratergal plates of female; B. sternal plate.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Description of a New Species of Hippobosca (*Diptera Pupipara*).

By G. A. H. BEDFORD, Section of Parasitology, Onderstepoort.

THE genus *Hippobosca* comprises nine species, including the new species described below.

Hippobosca martinaglia nov. sp. (Fig. 1.).

Five females and one male taken off impala, *Aepyceros melampus* (Lcht.), Bar R. Ranch, Swaziland, 25th June, 1934, and 1st July, 1935 (coll. G. Martinaglia and R. M. du Toit). *Holotype* a female.

A small species; length of wing 4·5 mm. *Head* about as wide at the occiput as at the fronto-clypeus, reddish-brown, the frontal stripe slightly darker; posterior margin of head fringed with minute, thick-set setae, and a long seta at the base of each eye; palpi dark brown, clothed with short setae of the same colour. *Thorax* reddish-brown, with a median dark band extending backwards almost to the transverse suture; this band is forked posteriorly, usually more so than in the figure, and in one specimen is completely divided down the middle by a narrow line; on each side of the posterior portion of the median band there is a dark transverse band. On each side beneath the transverse suture there is a narrow transverse dark band, and beneath this a small triangular spot, which is usually indistinct and may be absent. At each latero-anterior angle there are two short setae, one on each side slightly distad and nearer the meson; on each side above the base of the wing there are three very short, thick-set black setae, two more similar setae slightly above them and near the meson, and two larger setae slightly above them and near the meson, and two larger setae below them; on each side on the posterior margin there are five setae. Scutellum yellowish-white, fringed with short and a few long setae. On the venter there is a vertical dark band on each side between the fore and mid coxae. *Legs* pale reddish-brown, sparsely clothed with setae; those on the tibiae and tarsi darker. Ungues black. *Abdomen* reddish-brown with numerous pale setae. *Wings* hyaline with pale reddish-brown veins and short dark setae on the costa. Second longitudinal vein (R2+3) long, reaching beyond the apex of the first longitudinal vein (R1), but not extending to the anterior cross-vein.

This new species can be easily recognised by its pale colour and dark markings on the thorax and venter between the fore and mid coxae; also by the short thick-set setae on the thorax and pale scutellum.

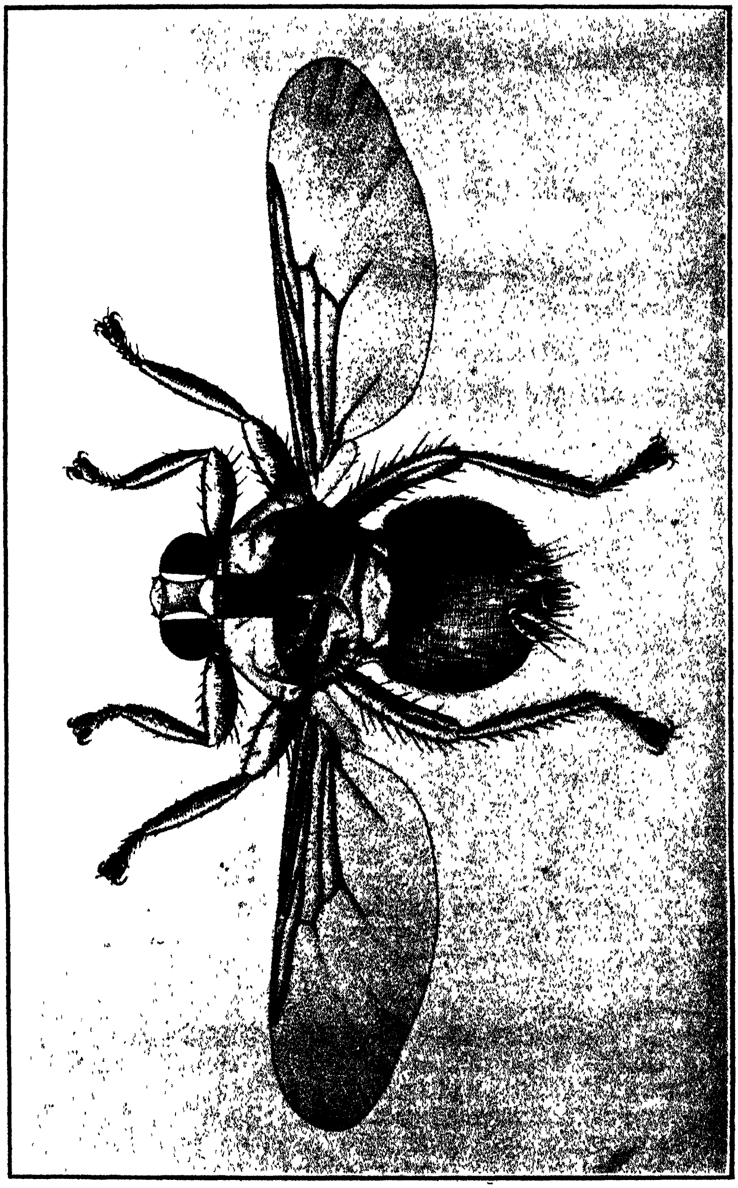


Fig. 1.

Hippobosca martinaglia nov. sp.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

A Synoptic Check-List and Host-List of the Ectoparasites found on South African Mammalia, Aves, and Reptilia.

(Supplement No. 1.)

By G. A. H. BEDFORD, Section of Parasitology, Onderstepoort.

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INTRODUCTION.

It has been considered advisable to bring the Synoptic Check-list of the Ectoparasites found on South African Mammalia, Aves and Reptilia, published in the 18th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, 1932, up to date by publishing supplements from time to time as necessity warrants, and this is the first of the series.

Not only is a number of new records reported upon in this paper, but it has also been found necessary to make alterations to the synonymy of a number of species.

References to the original check-list are recorded in this paper as follows:—

Bedford. *S. Afr. Ectoparasites*, p. ... (1932).

Order ACARINA.

Suborder ASTIGMATA.

Family ANALGESIDAE.

Genus Pterolichus Robin.

Pterolichus Bedford, *S. Afr. Ectoparasites*, p. 244 (1932).

Pterolichus ardeae (Canestrini).

Dermaleichus ardeae Canes.. *Atti Real. Ist. Veneto Sci., Lett. ed Art.* (5), V, p. 51 (1878).

Pterolichus (*Eupterolichus*) *ardeae* (Canes.) Canestrini & Kramer, *Demod. und Sarc.*, p. 41 (1899).

Recorded from *Botaurus stellaris* (Bittern) and other species of Ardeidae.

Pterolichus buchholzi (Canestrini). See *Ptiloxenus buchholzi* (Canestrini).

Pterolichus buchholzi fascigera Mégnin & Trouessart. See *Ptiloxenus fasciegera* (Mégnin & Trouessart).

Pterolichus columbi major Mégnin & Trouessart. See *Ptiloxenus major* (Mégnin & Trouessart).

Pterolichus nisi (Canestrini). See *Gabucinia nisi* (Canes.).

Pterolichus numenii (Canestrini). See *Avenzoaria numenii* (Canes.).

Pterolichus prozanae (Canestrini). See *Grallobia porzanae* (Canes.).

Pterolichus rallorum Robin. See *Grallobia rallorum* (Canes.).

Pterolichus rehbergi gracilis Mégnin & Trouessart. See *Philoxenus rehbergi gracilis* (Mégnin & Trouessart).

Pterolichus squatarolae (Canestrini). See *Avenzoaria squatarolae* (Canes).

Genus Avenzoaria Oudemans.

Avenzoaria Bedford, *S. Afr. Ectoparasites*, p. 248 (1932).

Avenzoaria numenii (Canestrini).

Pterolichus numenii (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 246 (1932).

Described from specimen taken off *Phaeopus phaeopus* (Whimbrel).

Avenzoaria ochropodus Hull.

Avenzoaria ochropodus Hull, *Trans. Northern Nat. Union*, p. 206, f. 1-3 (1934).

Described from specimens taken off Green Sandpiper, *Tringa erythropus* Vroeg. = *Totanus ochropus* L.

Avenzoaria squatarolae (Canestrini).

Pterolichus squatarolae (Canes.) Bedford. *S. Afr. Ectoparasites*, p. 248 (1932).

Described from specimens taken off Grey Plover, *Squatarola squatarola.*

Avenzoaria totani (Canestrini).

Avenzoaria totani (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 250 (1932).

Additional records: Hull (1934) records this species from *Crocethia alba* (Sanderling), and specimens have been taken off *Rhyacophilus glareola* (Wood Sandpiper) at Onderstepoort (coll. G.A.H.B.: det. C. D. Radford).

Genus PTILOXENUS Hull.

Ptiloxenus Hull, Trans. Northern Nat. Union, p. 202 (1934).

Genotype: Pterolichus major Mégnin and Trouessart.

Ptiloxenus bucholzi (Canestrini).

Pterolichus buchholzi (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 244 (1932).

Recorded taken off *Squatarola squatarola* (Grey Plover) and *Limosa limosa* (Black-tailed Godwit).

Ptiloxenus fasciger (Mégnin & Trouessart).

Pterolichus buchholzi fascigera Mégnin & Trouessart, Bedford, *S. Afr. Ectoparasite*, p. 245 (1932).

Recorded taken off *Arenaria interpres* (Trunstone), *Totanus totanus* (Redshank) and *Calidris canutus* (Knot).

Ptiloxenus major (Mégnin & Trouessart).

Pterolichus columbi major Mégn. & Trt., Bedford, *S. Afr. Ectoparasites*, p. 245 (1932).

Recorded taken off *crested* grebe, *Podiceps infuscata* (= *P. cristata*).

Ptiloxenus rehbergi gracilis (Mégnin & Trouessart).

Pterolichus rehbergi gracilis Mégn. & Trt., Bedford, *S. Afr. Ectoparasites*, p. 247 (1932).

Described from specimens taken off *Himantopus himantopus* (Black-wingel Stilt).

Ptiloxenus vanelli (Canestrini).

Dermaleichus vanelli Canes., *Atti Real. Ist. Venet oSci., Lett. ed Art.* (5), V, p. 62 (1878).

Proctophyllodes vanelli Canes., *Atti Soc. Veneto-Trent.*, VI, p. 37, pl. 1, f. 5 (1879).

Pterolichus (*Eupterolichus*) *vanelli* (Canes.) Canes. and Kramer, *Demod. und Sarc.*, p. 53 (1899).

Specimens have been taken off Crowned Lapwing, *Stephanibyx coronatus* (Bodd.) at Onderstepoort (coll. G.A.H.B.; det. C. D. Radford). Originally described from specimens taken off European Lapwing, *Vanellus vanellus* (= *V. cristatus*).

Genus GRALLOBIA Hull.

Grallobia Hull, *Trans. Northern Nat. Union*, p. 202 (1934).

This genus includes four species found on Rallidae.

Genotype: Dermaleichus porzanae Canestrini.

Grallobia porzanae (Canestrini).

Pterolichus porzanae (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 247 (1932).

Described from specimens taken off *Ortygometra porzana* (Spotted Crake).

Grallobia rallorum (Robin).

Pterolichus rallorum Robin Bedford, *S. Afr. Ectoparasites*, p. 247 (1932).

Described from specimens taken off *Crex crex* (Corn-crake).

Genus GABUCINIA Oudemans.

Gabucinia Bedford, *S. Afr. Ectoparasites*, p. 250 (1932).

Gabucinia nisi (Canestrini).

Pterolichus nisi (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 246 (1932).

Recorded taken off *Pygargus pygargus* (Montagu's Harrier) and *Pervis apivorus* (Honey Buzzard).

Genus EUSTATHIA Oudemans.

Eustathia Bedford, *S. Afr. Ectoparasites*, p. 251 (1932).

Chauliacia Bedford, *ibid.*, p. 251 (1932).

Chauliacia is considered to be a synonym of *Eustathia*. It included a single species—*securiger* Robin—found on *Micropus apus* (European Swift).

Genus HIRSTIA Hull.

Hirstia Hull, *The Vasculum*, XVII, iv, p. 145 (1931).

Hirstia chelidonis Hull.

Hirstia chelidonis Hull, *The Vasculum*, XVII, iv, p. 146 (1931). Described from specimens taken off *Chelidonaria urbica* (Mouse Martin) and *Hirundo rustica* (Swallow).

Genus SYRINGOBIA Trouessart & Neumann.

Syringobia Bedford, *S. Afr. Ectoparasites*, p. 255 (1932).

Syringobia calceata Trouessart.

Syringobia calceata Trt., Bedford, *S. Afr. Ectoparasites*, p. 256 (1932).

Males and females taken off *Thalasseus bergii* (Swift Tern), Swakopmund, South-West Africa (coll. R. D. Bradfield: det. C. D. Radford).

Syringobia chelopus Trouessart & Neumann).

Syringobia chelopus Trt. & Neu., Bedford, *S. Afr. Ectoparasites*, p. 256 (1932).

Hull (1934) has recorded this species from *Crocethia alba* (Sanderling).

Syringobia tricalcarata Trouessart & Neumann.

Syringobia tricalcarata Trt. & Neu., *Bull. Sci. France Belgique*, XIX, p. 345 (1888).

Syringobia tricalcarata Trt. & Neu., Canes. and Kramer. *Demod. und Sarc.*, p. 74 (1899).

Specimens have been taken off Three-banded Sandplover, *Afroxechus tricollaris* (Vieill.) at Onderstepoort (coll. G.A.H.B.; det. C. D. Radford). Originally described from specimens taken off *Charadrius dubius*.

Genus PTERONYSSUS Robin.

Pteronyssus, Bedford, *S. Afr. Ectoparasites*, p. 258 (1932).

Pteronyssus gracilipes Trouessart & Neumann. See *Leptosphyra gracilipes* (Trt. & Neu.).

Pteronyssus nuntiaeveris Berlese. See *Pteronyssoides nuntiaeveris* (Berlese).

Pteronyssus obscurus Berlese. See *Pteronyssoides obscurus* (Berlese).

Pteronyssus pallens Berlese. See *Pteronyssoides pallens* (Berlese).

Pteronyssus puffini (Buchholz).

Giebelia puffini (Buchh.) Bedford, *S. Afr. Ectoparasites*, p. 257 (1932).

Additional record: Specimens have been taken off *Thalasseus bergii* (Swift Tern) at Swakopmund, South-West Africa (coll. R. D. Bradfield; det. C. D. Radford).

Pternoyssus truncatus Trouessart. See *Pteronyssoides truncatus* (Trt.).

Genus PTERONYSSOIDES Hull.

Pteronyssoides Hull, *The Vasculum*, XVII, iv, p. 145 (1931).

Genotype: Pteronyssus striatus Robin.

The following species formally placed in the genus *Pteronyssus* are now included in this genus:—

Pteronyssoides nuntiaeveris (Berlese).

Pteronyssus nuntiaeveris Berl., Bedford, *S. Afr. Ectoparasites*, p. 258 (1932).

Pteronyssoides obscurus (Berlese).

Pteronyssus obscurus Berl., Bedford, *ibid*, p. 258 (1932).

Pteronyssoides pallens (Berlese).

Pteronyssus pallens Berl., Bedford, *ibid*, p. 258 (1932).

Pteronyssoides truncatus (Trouessart).

Pteronyssus truncatus Trt., Bedford, *ibid*, p. 258 (1932).

Genus MÉGNINIA Berlese.

Mégninia Bedford, *S. Afr. Ectoparasites*, p. 259 (1932).

Mégninia aestivalis Berlese. See *Pandalura aestivalis* (Berlese).

Mégninia aestivlais subintegra Berlese. See *Pandalura subintegra* (Berlese).

Mégninia columbae (Buchholz). See *Diplaegidia columbae* (Buchholz).

Genus PANDALURA Hull.

Pandalura Hull, *Trans. Northern Nat. Union*, p. 203 (1934).

Genotype: Dermaleichus strigisoti Buchholz.

Pandalura aestivalis (Berlese).

Mégninia aestivalis (Berl.) Bedford, *S. Afr. Ectoparasites*, p. 259 (1932).

Described from specimens taken off *Micropus apus* (European Swift).

Pandalura subintegra (Berlese).

Mégninia subintegra (Berl.) Bedford, *S. Afr. Ectoparasites*, *p*. 259 (1932).

Recorded taken off *Chelidonaria urbica* (House Martin) and *Riparia riparia* (European Sand Martin).

Genus DIPLAEGIDIA Hull.

Diplaegidia Hull, *Trans. Northern Nat. Union*, p. 203 (1934).

Genotype: Dermaleichus columbae Buchholz.

Diplaegidia columbae (Buchholz).

Mégninia columbae (Berl.) Bedford, *S. Afr. Ectoparasites*, p. 259 (1932).

Recorded from domestic and other pigeons in Europe.

Genus LEPTOSPHYRA Hull.

Leptosphyra Hull, *Trans. Northern Nat. Union*, p. 203 (1934).

Genotype: Analges centropodus Mégnin.

Leptosphyra gracilipes (Trouessart & Neumann).

Pteronyssus gracilipes (Trt. and Neu.), Bedford. *S. Afr. Ectoparasites*, p. 258 (1932).

Described from specimens taken off *Totanus totanus* (Redshank).

Genus ANALGES Nitzsch.

Analges Bedford, *S. Afr. Ectoparasites*, p. 260 (1932).

Analges bidentatus Giebel. See *Analgopsis bidentatus* (Giebel).

Analges bifidus (Nitzsch). See *Analgopsis bifidus* (Nitzsch).

Genus ANALGOPSIS Trouessart.

Analgopsis Trouessart, *Ann. Mag. Nat. Hist.*, Ser. 9, iv, p. 336 (1919).

Genotype: Acarus passerinus Linn.

Analgopsis bidentatus (Giebel).

Analges bidentatus (Giebel), Bedford, *S. Afr. Ectoparasites*, p. 260 (1932).

Recorded taken off the migrant, *Acrocephalus arundinaceus* (Great Reed Warbler), and other Passerines in Europe.

Analgopsis bifidus (Nitzsch).

Analges bifidus (Nitzsch) Bedford, *S. Afr. Ectoparasites*, p. 261 (1932).

Genus ALLOPTES Canestrini.

Alloptes Bedford, *S. Afr. Ectoparasites*, p. 262 (1932).

Alloptes lambda (Trouessart).

Pterocolus lambda Trouessart, *Bull. Soc. d'Etud. Sci. Angers*, XIV, p. 72 (1885).

Alloptes lambda (Trt.) Canestrini and Kramer, *Demod. und Sarc.*, p. 113 (1899).

Specimens have been taken off *Philomachus pugnax* (Ruff and Reeve) and *Rhyacophilus glareola* (Wood Sandpiper) at Onderstepoort (coll. G.A.H.B.; det. C. D. Radford). Originally described from *Nettapus auritus*, Madagascar.

Genus PTERODECTES Robin.

Pterodectes Bedford, *S. Afr. Ectoparasites*, p. 264 (1932).

Pterodectes ortygometrae (Canestrini). See *Brephosceles ortygometrae* (Canes.).

Pterodectes ortygometrae furciger (Trouessart). See *Brephosceles furcifer* (Trt.).

Genus BREPHOSCELES Hull.

Brephosceles Hull, *Transv. Northern Nat. Union*, p. 204 (1934).

Genotype: Pterolichus forficiger Mégnin and Trouessart.

Brephoceles ortygometrae (Canestrini).

Pterodectes ortygometrae (Canes.) Bedford, *S. Afr. Ectoparasites*, p. 265 (1932).

Recorded taken off *Ortygometra porzana* (Spotted Crake).

Brephosceles furcifer (Trouessart).

Pterodectes furcifer (Trt.) Bedford, *S. Afr. Ectoparasites*, p. 265 (1932).

Described from specimens taken off *Smutsornis africanus* (Two-banded Courser).

Genus PROCTOPHYLLODES Robin.

Proctophyllodes Robin, *Compt. Rend. hebdom. des Séan. Acad. Sci.*, LXVI, p. 786 (1868).

Genotype: Delmaleichus glandarinus C. L. Kock.

Proctophyllodes arcuaticaulis Trouessart.

Proctophyllodes arcuaticaulis Trouessart, *Bull. Soc. d'Étud. sci. Angers*, XVI, p. 148 (1886).

Proctophyllodes arcuaticaulis (Trt.) Canes & Kramer. *Demod. und Sarc.*, p. 118 (1899).

Specimens have been taken off S. African Tawny Pipit, *Anthus rugulus raalteni* (Lay.), Onderstepoort (coll. E. C. G. Bedford; det. C. D. Radford).

Suborder PROSTIGMATA.

Family PTERYGOSOMIDAE VITZTHUM.

This family includes a number of small mites parasitic on lizards. Lawrence (1935) has recorded 39 species from South Africa parasitic on lizards belonging to the families Geckonidae, Agamidae, Zonuridae, Gerrhosauridae and Lacertidae.

Keys to the species are not included here as Lawrence has given keys in his papers and these should be consulted.

Key to the South African Genera (after Lawrence).

1. A dorsal scute present 2
   A dorsal scute not present 3
2. Dorsum with few setae, these very long ... ... *Pimeliaphilus.*[1]
   Dorsum with numerous setae, these shorter ... ... *Geckobia.*
3. Apex of hypostome much enlarged, dorsum with few setae 4
   Hypostome subparallel; dorsum with numerous setae 5
4. Body longer than wide, size large, skin leathery *Ixodiderma.*
   Body much wider than long, size small, skin not leathery *Scaphothrix.*
5. Dorsum with a dense anterior patch of setae on each side of the mouth-parts, eyes absent ... ... *Pterygosoma.*
6. Dorsum without a dense anterior patch of setae on each side of the mouth-parts, eyes present ... ... *Zonurobia.*

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[1] No species has been found in South Africa up to the present, and it is unlikely that they occur in this region.

Genus IXODIDERMA Lawrence.

Ixodiderma Lawrence, *Parasitology*, XXVII, i, p. 22 (1935).

This genus includes three species parasitic on Zonuridae and Lacertidae.

Genotype: Ixodiderma inverta Lawrence.

1. **Ixodiderma inverta** Lawrence.

Ixodiderma inverta Lawrence, *Parasit.*, XXVII, i, pp. 24-29, t.f. 13-16 (1935).

Described from numerous adults and nymphs taken off *Pseudocordylus subviridis*, Belfast, Transvaal, also from *P. microlepidotus*, Table Mountain; *P. fasciatus*, Katberg, Coetzeesberg, Bedford, Butterworth, Grahamstown, Somerset East (Cape Province); *P. subviridis*, Lydenburg, Woodbush, Ermelo, Carolina, Wakkerstroom (Transvaal), Thaba Putsua, Maletsunyane Falls (Basutoland), Giants Castle (Natal); *Zonurus coeruleopunctatus*, Montague Pass, George; *Z. polyzonus*, Steinkop, Lamberts Bay, Phillipstown, Cradock (Cape Province); Aus, Krailuft (South-West Africa).

2. **Ixodiderma lacertae** Lawrence.

Ixodiderma lacertae Lawrence, *Parasit*, XXVII, i, pp. 29-30, t.f. 17 (1935).

Described from adults and nymphs taken off *Scapteira depressa*, locality unknown; also from *S. suborbitalis*, *S. knoxii* and *Tropidosaura gularis*, localities unknown, and from *Eremias undata*, Luderitzbucht, and *E. inornata*, Narudas Sud (South-West Africa). All the hosts belong to the family Lacertidae.

3. **Ixodiderma pilosa** Lawrence.

Ixoderma pilosa Lawrence, *Parsit*, XXVII, i, p. 30, f. 18 (1935).

Described from adults taken off *Pseudocordylus subviridis*, Drakensberg and Mont-aux-Sources, Basutoland.

Genus SCAPHOTHRIX Lawrence.

Scaphothrix Lawrence, *Parasitology*, XXVII, i, p. 20 (1935).

This genus includes a single species parasitic on Zonuridae.

1. **Scaphothrix convexa** Lawrence.

Scaphothrix convexa Lawrence, *Parasit.*, XXVII, i, pp. 21-22, t.f. 12 (1935).

Described from adults taken off *Zonurus polyzonus*, Vondeling, Cape Province; also from the same host species Kimberley; *Z. peersi*, Garies (Namaqualand) and *Z. cordylus*, Capetown.

Genus PTERYGOSOMA Peters.

Pterygosoma Peters, *Sitzber, Ges. Natf. Freunde, Berlin* (1849).

Eupterygosoma Trägardh, *Res. Swed. Zool. Exped. to Egypt and the White Nile, No.* 20, *Acariden*, p. 59 (1905).

Pterygosoma Lawrence, *Parasit.*, XXVIII, p. 20 (1936).

This genus includes fifteen species, twelve occurring on South African lizards belonging to the families Agamidae and Gerrhosauridae.

Genotype: Pterygosoma agamae Peters.

1. **Pterygosoma aculeatum** Lawrence.

Pterygosoma aculeatum Lawrence, *Parasit.*, XXVIII, p. 25, t.f. 20 (1936).

Described from adults taken off *Agama hispida aculeata*, Strydenburg, Cape Province; also from same host species, Kaotive, Central Kalahari; Kakamas; Kimberley and Fourteen Streams; and from *A. hispida distanti*, Pretoria, and Chisbawasha, Rhodesia.

2. **Pterygosoma agamae** Peters.

Pterygosoma agamae Peters, *Sitzber, Ges. Natf. Freunde*, Berlin (1849); *Hirst. Jour. Linn. Soc.* (*Zool.*), XXXVI, p. 194, f. 14 (1926); Lawrence, *Parasit.*, XXVIII, i, p. 25 (1936).

Recorded by Lawrence (1936) from *Agama Kirkii*, Mazoe, Chishawasha and Zimbabwe, Rhodesia; also from *Agama mossambica*, Mozambique.

3. **Pterygosoma armatum** Lawrence.

Pterygosoma armatum Lawrence, *Parasit.*, XXVIII, i, p. 30, t.f. 23 (1936).

Described from adults taken off *Agama hispida armata*, Weenen, Natal.

4. **Pterygosoma bedfordi** Lawrence.

Pterygosoma bedfordi Lawrence, *Parasit.*, XXVIII, i, p. 24, t.f. 16 *a*, *b*, 18 *a*, *b* (1936).

Described from adults, nymphs and larvae taken off *Agama atra atra*, Hermanus, Cape Province.

5. **Pterygosoma bicolor** Lawrence.

Pterygosoma bicolor Lawrence, *Parasit.*, XXVII, i, pp. 34-35, t.f. 20, 22 *c* (1935).

Described from adults taken off *Gerrhosaurus flavigularis*, Serowe, Bechuanaland; also from *G. nigrolineatus*, locality unknown, and *G. auritus*, Kaokoveld, South-West Africa.

6. **Pterygosoma gerrhosauri** Lawrence.

Pterygosoma gerrhosauri Lawrence, *Parasit.*, XXVII, i, pp. 33-34, t.f. 19 (1935).

Described from adults taken off *Gerrhosaurus validus*, Zululand; also from same host species, Plumtree, Rhodesia, and *G. major grandis*, Leydsdorp, Transvaal.

7. **Pterygosoma hirsti** Lawrence.

Pterygosoma hirsti Lawrence, *Parasit.*, XXVIII, i, p. 22, t.f. 17 *a-d* (1936).

Described from adults taken off *Agama atra atra*, Vondeling, Cape Province.

8. **Pterygosoma hystrix** Lawrence.

Pterygosoma hystrix Lawrence, *Parasit.*, XXVII, i, pp. 37-38, t.f. 21, 22 *a*, *b* (1935).

Described from adults found on venter scales of belly and tail of *Gerrhosaurus flavigularis;* Grahamstown, Cape Province.

9. **Pterygosoma longipalpe** Lawrence.

Pterygosoma longipalpe Lawrence, *Parasit.*, XXVIII, i, p. 25, t.f. 19 *a*, *b* (1936).

Described from adults taken off *Agama atra atra*, Namaqualand; also from same host, Vondeling, and from *Agama hispida aculeata*, Murraysburg.

10. **Pterygosoma melanum** Hirst.

Pterygosoma melanum Hirst, *Journ. Linn. Soc.* (Zool.), XXXVI, p. 196, f. 16 (1926); Lawrence, *Parasit.*. XXVIII, i, pp. 21-22 (1936).

Recorded by Lawrence (1935) from the following hosts: *Agama atra atra*, Cradock, Beaufort West, Kimberley, Fourteen Streams, Victoria West, Van Rhynsdorp, Hanover, Phillipstown, Hermanus, St. James, Table Mountain, and Cape Point, Cape Province; Garies, Namaqualand; Rustenburg, Pretoria, Rosslyn, Maquassie, near Wolmaransstad, Transvaal; Vryburg, Bechuanaland; Maseru, Basutoland, and Luderitzbucht, South-West Africa; also from *Agama anchietae knobelii*, Aus, South-West Africa.

11. **Pterygosoma transvaalensis** Lawrence.

Pterygosoma transvaalensis Lawrence, *Parasit.*, XXVIII, i, pp. 28-29, t.f. 22 (1936).

Described from adults taken off *Agama atricollis*, Mariepskop, Eastern Transvaal; also from the same host species, Weenen, Natal.

12. **Pterygosoma triangulare** Lawrence.

Pterygosoma triangulare Lawrence, *Parasit.*, XXVIII, i, p. 28, t.f. 21 (1936).

Described from adults taken off *Agama hispida brachyura*, Bitterfontein, Namaqualand; also from same host, unknown locality, and from *A. hispida hispida*, Darling and Port Nolloth.

Genus ZONUROBIA Lawrence.

Zonurobia Lawrence, *Parasit.*, XXVII, i, p. 4 (1935).

This genus includes nine species and five varieties found on South African lizards belonging to the family *Zonuridae*.

Genotype: Zonurobia cordylensis.

1. **Zonurobia circularis circularis** Lawrence.

Zonurobia circularis Lawrence, *Parasit.*, XXVII, i, pp. 8-9, t.f. 1 *c*, *d*, 4 (1935).

Described from adults taken off *Platysaurus guttatus*, Salisbury, S. Rhodesia; also from the same host species, Plumtree, Chisawasha, Zimbabwe (Southern Rhodesia); and Pietersburg, Mariepo, Shiliowane, Nylstroom (Transvaal); and four specimens from *Zonurus jonesi* which probably constitute a variety.

Zonurobia circularis capensis Lawrence.

Zonurobia circularis, var. *capensis*, Lawrence, *Parasit.*, XXVII, i, p. 10 (1935).

Described from specimens taken off *Platysaurus capensis*, Kamieskroon, Namaqualand.

Zonurobia circularis latior Lawrence.

Zonurobia circularis latior Lawrence, *Parasit.*, XXVII, i, pp. 9-10 (1935).

Described from specimens taken off *Zonurus vittifer*, Belfast Potgietersrust, Haenertzburg, Middelburg (Transvaal), and Ubombo (Zululand); also from *Zonurus breyeri*, Waterberg Mountains, Transvaal.

Zonurobia circularis longipilis Lawrence.

Zonurobia circularis, var. *longipilis*, Lawrence, *Parasit.*, XXVII, i, pp. 10-11 (1935).

Described from specimens taken off *Platysaurus guttatus*, Blyde River, Transvaal.

Zonurobia circularis spiniventer Lawrence.

Zonurobia circularis, var. *spiniventer*, Lawrence, *Parasit.*, XXVII, i, p. 10, t.f. 5 (1935).

Described from specimens taken off *Platysaurus guttatus minor*, Nylstroom and Waterberg District; Transvaal.

Zonurobia circularis transvaalensis Lawrence.

Zonurobia circularis, var. *transvaalensis*, Lawrence, *Parasit.*, XXVII, i, p. 11 (1935).

Described from specimens taken off *Platysaurus wilhelmi*, Olifants River, Eastern Transvaal.

2. **Zonurobia cordylensis** Lawrence.

Zonurobia cordylensis Lawrence, *Parasit.*, XXVII, i, pp. 5-7, t.f. 1 *a*, *b*, 2 (1935).

Described from adults and nymphs taken off *Zonurus cordylus*, Table Mountain, East London, Stutterheim, George, and Hogsback, in the Cape Province; also from specimens from the same host, Smithfield, Orange Free State, which may constitute a variety.

3. **Zonurobia debilipes** Lawrence.

Zonurobia debilipes Lawrence, *Parasit.*, XXVII, i, pp. 13-15, t.f. 1 *i*, 7 (1935).

Described from adults taken off *Zonurus warreni*, Ubombo, Zululand.

4. **Zonurobia montana** Lawrence.

Zonurobia montana Lawrence, *Parasit.*, XXVII, i, pp. 19-20, t.f. 11 (1935).

Described from specimens taken off *Zonurus namaquensis*, Karasberg Mountains, South-West Africa.

5. **Zonurobia polyzonensis** Lawrence.

Zonurobia polyzonensis Lawrence, *Parasit.*, XXVII, i, pp. 7-8, t.f. 1 *b*, *g*, 3 (1935).

Described from specimens taken off *Zonurus polyzonus*, unknown locality; also from same host species from Kimberley, Vondeling, Darling, Leliefontein (Namaqualand), Kuruman, Fourteen Streams, Van Rhynsdorp, and four specimens from *Zonurus peersi*, Kamiesburg (Namaqualand).

6. **Zonurobia sanguinea** Lawrence.

Zonurobia sanguinea Lawrence, *Parasit.*, XXVII, i, pp. 16-18, t.f. 9 (1935).

Described from specimens taken off *Zonurus coeruleopunctatus*, Montagu Pass, George.

7. **Zonurobia semilunaris** Lawrence.

Zonurobia similunaris Lawrence, *Parasit.*, XXVII, i, pp. 11-13, t.f. 1 *e*, *f*, 6 (1935).

Described from specimens taken off *Pseudocordylus microlepidotus*, unknown locality; also from *P. fasciatus*, Bedford and Grahamstown, Cape Province.

8. **Zonurobia subquadrata** Lawrence.

Zonurobia subquadrata Lawrence, *Parasit.*, XXVII, i, pp. 18-19, t.f. 10 (1935).

Described from specimens taken off *Zonurus robertsi*, between Nieuwoudville and Van Rhynsdorp, and at Klaver, C.P.

9. **Zonurobia transvaalica** nom. nov. (Lawrence).

Zonurobia transvaalensis Lawrence, *Parasit.*, XXVII, i, p. 15, t.f. 8 (1935), *nec*. Lawrence, p. 11, 1935.

Described from specimens taken off *Zonurus vandami*, Leydsdorp, Transvaal; also from *Z. vandami perkoensis*, Eastern Transvaal; *Z. barbertonensis depressus*, Louis Trichardt; and *Z. laevigatus*, Entabeni, Zoutpansberg, Transvaal.

Genus GECKOBIA Mégnin.

Geckobia Mégnin, *Bull. Soc. Ent. Fr.*, Ser. 5, p. 8 (1878).
Geckobia Lawrence, *Parasit.*, XXVIII, i, p. 2 (1936).

Lawrence (1936) has recorded fourteen species from South African Geckonidae.

1. **Geckobia australis** Hirst.

Geckobia australis Hirst, *Jour. Linn. Soc.* (Zool.), XXXVI, p. 176, f. 3 (1926).
Geckobia australis (Hirst) Lawrence, *Parasit.*, XXVIII, i, p. 4 (1936).

Recorded found on *Lygodactylus capensis capensis*. The types were from Beira, Portuguese East Africa, and Lawrence (1936) has recorded it from the following localities in the Transvaal: Hectorspruit, Komatipoort, Mariepskop, Palmyraville, Zoutpansberg, Roodepoort, Pretoria District, Rustenburg and Vygeboompoort; also from Salisbury and unknown locality, Rhodesia.

2. **Geckobia hemidactyli** Lawrence.

Geckobia hemidactyli Lawrence, *Parasit.*, XXVIII, i, p. 14, t.f. 11 (1936).

Described from specimens taken off *Hemidactylus tasmani*, Driefontein, Southern Rhodesia; also from the same host species, Kutama, Southern Rhodesia; *H. mabouia*, Lourenço Marques, and Zambi, Belgian Congo.

3. **Geckobia hewitti** Lawrence.

Geckobia hewitti Lawrence, *Parasit.*, XXVIII, i, pp. 8-10, t.f. 7 (1936).

Described from specimens taken off *Pachydactylus maculatus*, Resolution Halt, Grahamstown; also from same host, unknown locality.

4. **Geckobia homopholis** Lawrence.

Geckobia homopholis Lawrence, *Parasit.*, XXVIII, i, p. 15, t.f. 1, 12 (1936).

Described from specimens taken off *Homopholis wahlbergii*, Bulawayo and Salisbury, Rhodesia; Geelhoutkop, Waterberg District, and Waterval Boven, Transvaal.

5. **Geckobia karrooica** Lawrence.

Geckobia Karrooica Lawrence, *Parasit.*, XXVIII, i, p. 18, t.f. 15 (1936).

Described from specimens taken off *Oedura Karrooica wilmoti*, Tarkastad.

6. **Geckobia namaquensis** Lawrence.

Geckobia namaquensis Lawrence, *Parasit.*, XXVII, p. 10, t.f. 8 (1935).

Described from specimens taken off *Pachydactylus namaquensis*, Garies and Steinkopf, Namaqualand; also from *Pachydactylus capensis gariesensis*, Garies.

7. **Geckobia natalensis** Lawrence.

Geckobia natalensis Lawrence, *Parasit.*, XXVII, p. 8, t.f. 6 (1935).

Described from specimens taken off *Lygodactylus capensis capensis*, Umhlatu, Natal, and Mozambique.

8. **Geckobia oedurae** Lawrence.

Geckobia oedurae Lawrence, *Parasit.*, XXVIII, i, p. 6, t.f. 6 (1936).

Described from specimens taken off *Oedura transvaalica platyceps*, Empandeni, near Salisbury, and Driefontein, Southern Rhodesia.

9. **Geckobia ovambica** Lawrence.

Geckobia ovambica Lawrence, *Parasit.*, XXVIII, i, p. 4, t.f. 3 (1936).

Described from two specimens taken off *Rhoptropus barnardi*, Kunene River, South-West Africa.

10. **Geckobia pachydactyli** Lawrence.

Geckobia pachydactyli Lawrence, *Parasit.*, XXVIII, i, pp. 12-14, t.f. 10 (1936).

Described from specimens taken off *Pachydactylus bibronii, bibronii*, Bitterfontein, Namaqualand; also from the same host, Aus and Oup River, South-West Africa, and Leliefontein, Namaqualand.

11. **Geckobia phyllodactyli** Lawrence.

Geckobia phyllodactyli Lawrence, *Parasit.*, XXVIII, i, p. 15, t.f. 2, 13 (1936).

Described from adults, nymph and larva taken off *Phyllodactylus porphyreus*, Hermanus and Capetown.

12. **Geckobia rhoptropi** Lawrence.

Geckobia rhoptropi Lawrence, *Parasit.*, XXVIII, i, pp. 11-12, t.f. 9 (1936).

Described from specimens taken off *Rhoptropus ocellatus*, Leliefontein, Namaqualand; also from *Lygodactylus bradfieldi*, Okahandja, South-West Africa.

13. **Geckobia tasmani Lawrence.**

Geckobia tasmani Lawrence, *Parasit.*, XXVIII, i, p. 6, t.f. 4 (1936).

Described from specimens taken off *Hemidactylus tasmani*, Chishawasha, near Salisbury, Rhodesia.

14. **Geckobia transvaalensis** Lawrence.

Geckobia transvaalensis Lawrence, *Parasit.*, XXVIII, i, pp. 17-18, t.f. 14 (1936).

Described from specimens taken off *Lygodactylus capensis capensis*, Eastern Transvaal; also from *Pachydactylus capensis affinis*, Plumtree, Southern Rhodesia; *P. capensis capensis*, Kimberley and Vryburg; *P. capensis tigrinus*, Zoutpansberg; *Chondrodactylus angulifer*, Kanus Siding, South-West Africa; and doubtful records from *Pachydactylus punctatus* and *P. capensis formosus*, unknown localities.

Suborder MESOSTIGMATA.

Family DERMANYSSIDAE.

Genus Liponyssus Kolenati.

Liponyssus biscutatus Hirst.

♀ *Liponyssus biscutatus* Hirst, *Proc. Zool. Soc. Lond.*, p. 779, t.f. 21, 22 (1921).

Described from specimens taken off Cardinal Woodpecker, *Dendropicus fuscescens* (= *D. cardinalis*), South Africa. Two females have also been taken off *D. fuscescens transvaalensis*, Buffelsdraai, Transvaal, 26th March, 1916 (det. C. D. Radford).

Liponyssus bursa (Berlese).

Leiognathus bursa Berlese, *Bull. Soc. ent. Ital.*, XX, p. 208, pl. 9, f. 6 (1888).

♂ ♀ *Leiognathus morsitans* Hirst, *Bull. Ent. Res.*, VI, pp. 56-58, t.f. 1-3 (1915).

Liponyssus bursa (Berlese) Hirst, *Mites Injurious to Domestic Animals*, pp. 89-90, t.f. 66, 67A, 68A, 69A, 70 (1922).

Liponyssus bursa (Berlese) Bedford, *S. Afr. Ectoparasites*, p. 272 (1932).

Specimens have been taken off South African Tawny Pipit. *Anthus rufulus raalteni* (Lay.), Onderstepoort (coll. E. C. G. Bedford; det. C. D. Radford).

Liponyssus flavus (Kolenati).

Males and females taken off Cardinal Woodpecker, *Dendropicus fuscescens transvaalensis* Rbts., Buffelsdraai, Transvaal, 26th March, 1916 (det. C. D. Radford). This species was described from specimens taken off bats, and Hirst (1921) has recorded it from noctule bat, Aylesford, Kent, England.

Liponyssus viator Hirst.

♀ *Liponyssus viator* Hirst, *Proc. Zool. Soc. Lond.*, p. 775, t.f. 20 (1921).

Described from specimens taken off Indian Swift, *Colletoptera* (= *Cypselus*) *affinis*, Calcutta; also from Woodpecker, *Gecinus vaillanti*, Tangier. Specimens have been taken off Cape Black-headed Sparrow, *Passer melanurus melanurus*, Onderstepoort (coll. G.A.H.B.; det. C. D. Radford).

Superfamily IXODOIDEA.

Family ARGASIDAE.

Genus ARGAS Latreille.

Argas brumpti Neumann.

Ad. ♂ *Argas brumpti* Neumann, *Archiv. de Parasit.*, XI, pp. 224-229, f. 9-14 (1907); Nuttall, Warburton, Cooper and Robinson, *Ticks: Mon. Ixod*, I, pp. 30-33, 95, f. 38-47 (1908); Neumann, *Das Tierreich, Ixodidae*, pp. 121-122 (1911).

L. *Argas brumpti* (Neu.), Cunliffe, *Parasit.*, VI, pp. 379-381, t.f. 1 (1914).

Adults and nymphs have been found at Gemsbok Plain, Oup River, Kalahari (coll. V. FitzSimons), and one adult has been received from Dr. R. F. Lawrence found under a stone at Lekkersing, Richtersveld; also engorged larvae, which I refer to this species, from a lizard, *Gerrhosaurus validus*, Mara, N.W. Transvaal.

This species was originally described from specimens collected in Somaliland. They attacked man in dusty sheltered hollowed out places beneath overhanging rocks into which opened porcupine burrows. Cunliffe (1914) recorded specimens from Kenya Colony. They were found in dust of an antheap, and also reported to be found in dust where big game roll. King (*Bull. Ent. Res*, VI, pp. 191-193, 1915) and Ruttledge (*Bull. Ent. Res.*, XXI, p. 273, 1930) have recorded this species from the Sudan; the former found adults and nymphs in crevices between rocks and in caves, and the latter larvae on the ground lizard, *Agama colonorum*. King has reared larvae on a guinea-fowl, *Numida meleagris*, but failed to feed them on fowls, sparrows, pigeons, doves and bats.

Argas moubata Murray.

Argas moubata (Murray) Bedford, *S. Afr. Ectoparasites*, p. 280 (1932).

Additional record: Numerous specimens collected in a pig-stye at Doornkraal, Pietersburg District, Northern Transvaal, March, 1934 (coll. G. V. O. Lund). Attempts to transmit *Aegyptianella pullorum* to fowls with this species failed (Bedford and Coles, 1933).

Argas persicus (Oken).

Argas persicus (Oken), Bedford, *S. Afr. Ectoparasites*, p .281 (1932).

This species has been proved by Bedford and Coles (1933) to transmit *Aegyptianella pullorum* to domestic fowls.

Family IXODIDAE.

Genus IXODES Latreille.

Ixodes pilosus Koch.

Ixodes pilosus (Koch) Bedford, *S. Afr. Ectoparasites*, p. 285 1932).

Additional record: Specimens sent by Dr. R. F. Lawrence taken off *Leptailurus serval* Schr. (Serval Cat), Pietermaritzburg, Natal.

Genus HAEMAPHYSALIS Koch.

Haemaphysalis aciculifer Warburton.

Haemaphysalis aciculifer (Warb.) Bedford, *S. Afr. Ectoparasites*, p. 289 (1932).

Additional record: Males and females taken off *Leptailurus serval* Schr. (Serval Cat), Pietermaritzburg, Natal (sent by Dr. R. F. Lawrence).

Haemaphysalis leachii (Audouin).

Haemaphysalis leachii (Aud.) Bedford, *S. Afr. Ectoparasites*, p. 289 (1932).

Additional record: Adults taken off the same host as the above species.

Haemaphysalis silacea Robinson.

Haemaphysalis silacea (Robinson) Bedford, *S. Afr. Ectoparasites*, p. 290 (1932).

Additional record: Adults taken off *Tragelaphus sylvaticus* (Bushbuck), Mount Edgecombe, Natal, July, 1934.

Genus RHIPICEPHALUS Koch.

Rhipicephalus simpsoni Nuttall.

♂ ♀ *Rhipicephalus simpsoni* Nuttall, *Parasit.*, III, iv, pp. 413-416, t.f. 6, 7 (1910).

A male recorded by Bedford (1932) as *R. simus* C. L. Koch from *Thryonomys swinderianus variegatus* (Natal Cane-rat), Nylstroom, Transvaal, proves to be this species. Adults have also been received from Mr. C. H. E. Hopkins taken off *T. swinderianus*, N'buya, Uganda. Originally described from adults taken off a large rodent in S. Nigeria.

Rhipicephalus simus C. L. Koch.

Rhipicephalus simus (C. L. Koch) Bedford, *S. Afr. Ectoparasites*, p. 298 (1932). See *Rhipicephalus simpsoni* (Nuttall).

Genus HYALOMMA Koch.

Hyalomma aegyptium aegyptium (Linnaeus).

Hyalomma aegyptium aegyptium (Linn.) Bedford, *S. Afr. Ectoparasites*, p. 301 (1932).

Additional record: One female taken off *Atelerix frontalis* (Hedgehog). Springvale, South-West Africa (coll. E. Meyer).

Hyalomma aegyptium impressum Koch.

Hyalomma aegyptium impressum (Koch) Bedford, *S. Afr. Ectoparasites*, p. 302 (1932).

Additional record: One larva off *Bhuchanga adsimilis* (Fork-tailed Drongo), Onderstepoort.

Genus AMBLYOMMA Koch.

Amblyomma hebraeum Koch.

Amblyomma hebraeum Koch (Bedford), *S. Afr. Ectoparasites*, p. 304 (1932).

Additional record: Adults taken off *Aepyceros melampus* (Impala), Bar R Ranch, Swaziland (coll. G. Martinaglia).

Amblyomma petersi Karsch.

Amblyomma petersi (Karsch) Bedford, *S. Afr. Ectoparasites*, p. 306 (1932).

Additional record: One female received from Mr. C. Kent taken off tortoise, Melmoth, Zululand.

Genus APONOMMA Neumann.

Aponomma laeve capensis Neumann.

Aponomma laeve capensis (Neu.) Bedford, *S. Afr. Ectoparasites*, p. 308 (1932).

Additional record: Adults taken off *Acontias plumbeus* (Blind Lizard). Sangwana District, Zululand (coll. H. W. Bell Marley).

Aponomma neglectum S. and L. F. Hirst.

♂ ♀ *Amblyomma* (*Aponomma*) *neglectum* S. and L. F. Hirst, *Ann. Mag. Nat. Hist.* (8), VI, p. 305, t.f. 7-8 (1910).

Described from adults taken off *Varanus albigularis*, Deelfontein, C.P. The male has three metallic green spots on the scutum and the female one.

Suborder MALLOPHAGA.

Superfamily ISCHNOCERA.

Family PHILOPTERIDAE.

Genus DEGEERIELLA Neumann.

Degeeriella Bedford, *S. Afr. Ectoparasites*, p. 319 (1932).

Degeeriella annulata (Denny).

Nirmus annulatus Burmeister, *Handb. der. Ent.*, ii, p. 428 (1838); no description; Denny, *Mon. Anoplur. Brit.*, p. 132, pl. 8, f. 5 (1842).

Nirmus oedicnemi Denny, *ibid.*, p. 138, pl. 7, f. 8 (1842).

Nirmus annulatus Nitzsch in Giebel, *Zeit. f. ges. Nat.*, XIII, p. 311 (1861). Giebel, *Insecta Epizoa*, p. 159, pl. 5, f. 9, 10 (1874).

Docophorus annulatus Piaget, *Les Pédiculines*, p. 664, pl. 54, f. 5 (1880).

Philopterus annulatus Harrison, *Parasit.*, IX, i, p. 88 (1916).

Degeeriella oedicnemi Harrison, *ibid.*, IX, i, p. 119 (1916).

Previously recorded from the Stone Curlew, *Oedicnemus oedicnemus* (= *O. crepitans*); also by Piaget (1880) from *O. magnirostris*. Specimens have been taken off *Oedicnemus vermiculatus* (Water Thickknee), Rustenburg District, Transvaal (coll. W. Powell). Through the kindness of Colonel Meinertzhagen I have been able to compare these with specimens taken off the type host, *O. oedicnemus*, Suffolk, England.

Piaget was correct in considering Denny's *D. oedicnemi* to be immature specimens of *D. annulata*, but it is difficult to understand how he came to place this species in the genus *Docophorus*.

Degeerialla birostris (Giebel).

Nirmus birostris Giebel, *Insecta Epizoa*, p. 174 (1874).

Nirmus gloriosus Kellog & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 467, pl. 29, f. 1 (1902), part; Kellog, *Trans. Am. Ent. Soc.*, XXXII, p. 313 (1906); Kellog & Paine, *Ent. News*, XXI, p. 125 (1910); Kellog & Mann, *Ent. News*, XXIII, p. 58 (1912).

Degeeriella gloriosa Ferris, *Bern. P. Bishop Mus., Honolulu*, Bull. 98, pp. 68-69, t.f. 18A-E (1932); Bedford, *S.A. Ectoparasites*, p. 322 (1932).

The types (males) of *D. birostris* were taken off *Sterna* sp. = ? *fuliginosa*. It has also been recorded as *D. gloriosa* from *Sterna fuliginosa*, *S. anathaeta*, *Anous stolidus*, and certain land birds (abnormal hosts), Galapagos and Revillagigedo Islands; *Sterna lunata*, Laysan Is., and *Sterna fuscata*, Marquesas. Specimens have been taken off *Sterna fuliginosa*, Eshowe, Zululand (coll. B. De Meillon).

Degeerialla colymbina (Scopoli). See *Esthiopterum colymbina* (Scopoli).

Degeerialla emarginata (Kellogg & Chapman). See *D. separata* (Kellogg & Kuwana).

Degeeriella gloriosa (Kellogg & Kuwana). See *D. birostris* (Giebel).

Degeeriella separata (Kellogg & Kuwana).

Nirmus gloriosus Kellog & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 467 (1902), Part ...

Nirmus separatus Kellog & Kuwana, *ibid.*, IV, p. 472, pl. 29, f. 6 (1902). Kellog, *Trans. Am. Ent. Soc.*, XXXII, p. 317 (1906), part; Uchida, *Annot. Zool. Japan*, IX, p. 484 (1918).

Nirmus gloriosus var. *emarginatus* Kellog & Chapman, *Jour. New York Ent. Soc.*, X, p. 159 (1902).

Degeeriella separata (K. & K.) Ferris, *Bern. P. Bishop Mus., Honolulu*, pp. 69-70, t.f. 19A-E (1932).

This species has, as Ferris has shown, been confused with *D. birostris*. It has been taken off *Anous stolidus*, *Sterna fuliginosa* and certain land birds (abnormal hosts), Galapagos and Revillagigedo Islands: also off *Anous stolidus*, Hawaii and Marquesas.

Degeeriella scolopacis (Denny). See *Rhynonirmus scolopacis* (Denny).

Degeeriella vanelli (Denny).

Nirmus vanelli Denny, *Mon. Anoplur. Brit.*, p. 128, pl. 7, f. 6 (1842).

Described fro mspecimens taken off *Squatarola* (= *Vanellus*) *squatarola* (Grey Plover), a migrant to South Africa. Harrison (1916) sank *D. vanelli* as a synonym of *D. juncea* (Scopoli) found on *Vanellus vanellus*, but it is a distinct species.

Genus Rhynonirmus Thompson.

Rhynonirmus Thompson, *Parasitology*, XXVII, ii, p. 281 (1935).

This genus includes three species found on Scolopacidae. *Genotype: Lipeurus infuscatus* Osborn.

Rhynonirmus scolopacis (Denny).

Degeeriella scolopacis (Denny) Bedford, *S. Afr. Ectoparasites*, p. 327 (1932).

Rhynonirmus scolopacis (Denny) Thompson, *Parasit.*, XXVII, ii, p. 284, t.f. 2A-B, 4C (1935).

Recorded taken off *Capella* (= *Scolopax*) *gallinago* in Europe, and *Capella nigripennis* in South Africa.

Genus Goniodes Nitzsch

Goniodes numidae Mjöberg.

Goniodes numidae (Mjöberg) Bedford, *S. Afr. Ectoparasites*, p. 330 (1932).

Additional record: Specimens taken off *Numida coronata*, Vryburg, C.P.

Genus Goniocotes Burmeister.

Goniocotes gigas Taschenberg.

Goniocotes gigas (Taschenb.) Bedford, *S. Afr. Ectoparasites*, p. 332 (1932).

Additional record: Specimens taken off *Numida coronata*, Vryburg, C.P.

Goniocotes hologaster Nitzsch.

Goniocotes hologaster (Nitzsch) Bedford, *S. Afr. Ectoparasites*, p. 331 (1932).

Additional record: Specimens taken off *Numida coronata*, Vryburg, C.P

Genus PECTINOPYGUS Mjöberg.

Pectinopygus gracilicornis (Piaget).

♂ *Lipeurus gracilicornis* Piaget, *Les Pédiculines*, p. 309, pl. 25, f. 6 (1880), part.

♂ *Lipeurus gracilicornis*, var. *majus*, Kellogg, *Occ. Papers, California Acad. Sci.*, VI, p. 30, pl. 3, f. 5 (1899), part; Kellogg & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 477 (1902), part; Kellogg, *Trans. Amer. Ent. Soc.*, XXXIII, p. 321 (1906).

♂ *Esthiopterum gracilicornis* (Piaget) Ferris, *Bern. P. Bishop Mus., Honolulu*, Bull. 98, pp. 61-62, t.f. 13A-E (1932).

Ferris has shown this species to have been confused with *P. sulae* (Rudow). It has been recorded from *Fregata minor* and *F. aquila*, the former occurring on the South African coast.

Pectinopygus sulae (Rudow).

Lipeurus sulae Rudow, *Zeit. f. ges. Naturw.*, XXXVI, p. 134 (1870).

♀ *Lipeurus gracilicorns* Piaget, *Les Pédiculines*, p. 309 (1880), part.

♀ *Lipeurus gracilicornis*, var. *major*, Kellogg, *Occ. Papers, California Acad. Sci.*, VI, p. 30 (1899), part; Kellogg & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 477 (1902), part; Kellogg, *Trans. Am. Ent. Soc.*, XXXII, p. 319 (1906), part.

♂ *Lipeurus helleri* Kellogg & Küwana, *Proc. Wash. Acad. Sci.*, IV, p. 479, pl. 30, f. 3 (1902); Kellogg, *Trans. Amer. Ent. Soc.*, XXXII, p. 319 (1906), part.

Lipeurus potens Kellogg & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 477 (1902), part; Kellog, *Trans. Amer. Ent. Soc.*, XXXII, p. 319 (1906), part.

♀ ♂ *Pectinopygus sulae* (Rudow) Ferris, *Bern. P. Bishop Mus., Honolulu*, Bull. 98, pp. 64-67, t.f. 16A-B, 17A-D (1932).

This species has, as Ferris has shown, been confused with both *P. gracilicornis* and *P. potens*. It has been found on *Fregata minor*, which occurs on the South African coast; also on *Fregata aquila*, *Sula* spp. and several abnormal hosts.

Genus ESTHIOPTERUM Harrison.

Esthiopterum Bedford, *S. Afr. Ectoparasites*, p. 337 (1932).

Esthiopterum anseris (Linn.). See *Anaticola anseris* (Linn.).

Esthiopterum ardeae (Linn.). See *Anaticola ardea* (Linn.).

Esthiopterum asymmetricum (Rudow). See *Anaticola asymmetrica* (Rudow).

Esthiopterum capitatum (Piaget). See *Anaticola capitata* (Piaget).

Esthiopterum ciconiae (Linn.). See *Ardeicola ciconia* (Linn.).

Esthiopterum colymbina (Scopoli).

Degeeriella colymbina (Scop.) Bedford, *S. Afr. Ectoparasite*, p. 320 (1932).

This species must be placed in *Esthiopterum* and not in *Degeeriella*. It cannot remain in *Esthiopterum*, however, and eventually a new genus will have to be erected for it.

Esthiopterum concinnum (Kellogg and Chapman).

Lipeurus concinnus Kellogg & Chapman, *Occ. Papers, California Acad. Sci.*, VI, pp. 97-99, pl. 7, f. 2 (1899).

Described from specimens taken off *Diomedea albatrus*. Specimens have also been taken off *Nealbatrus chlororhynchus* (Yellow-billed Mollymawk), Capetown, C.P. (coll. R. F. Lawrence).

Esthiopterum crassicorne (Scop.). See *Anaticola crassicornis* (Scop.).

Esthiopterum diversum (Kellogg).

Esthiopterum diversum (Kellogg). *S. Afr. Ectoparasites*, p. 338 (1932).

Additional record: Recorded by Kellogg & Chapman (1899) taken off *Neonectris* (= *Puffinus*) *griseus* (Sooty Shearwater).

Esthiopterum genitale (Piaget). See *Ardeicola genitalis* (Piaget).

Esthiopterum giganticola (Kellogg). See *E. tricolor* (Piaget).

Esthiopterum lepidum (Nitzsch). See *Ardeicola lepida* (Nitzsch).

Esthiopterum leucoproctum (Nitzsch). See *Ardeicola leucoprocta* (Nitzsch).

Esthiopterum maculatum (Nitzsch). See *Ardeicola maculata* (Nitzsch).

Esthiopterum obscurum (Rudow).

Esthiopterum obscurum (Rudow) Bedford, *S. Afr. Ectoparasites*, p. 340 (1932).

Both *E. tricolor* (Piaget) and *E. lepturus* (Enderlein) have been recorded as synonyms of this species, but they are the same as *E. giganticola* (Kellogg). *E. obscurum*, of which *E. melanocnemis* (Giebel) and *E. gaini* (Neumann) are synonyms, has only been found on *Macronectes gigantea* (Giant Petrel).

Esthiopterum pederiforme (Dufour).*

Philopterus pederiformis Dufour, *Ann. Soc. Ent. Fr.*, IV, p. 676, pl. 21, f. 4 (1834).

Metapeuron laeve Rudow, *Zeit. f. ges. Naturw.*, XXXV, p. 140 (1870).

Lipeurus angulicollis Giebel, *Ann. Mag. Nat. Hist.*, XVII, p. 388 (1876).

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* Thompson [*Ann. Mag. Nat. Hist.* (10), xvi, p. 485, 1935] has recently erected the genus *Episbates* for the reception of this species.

Lipeurus breviceps Piaget, *Tijd. v. Ent.*, XXXIII, p. 243, pl. 9, f. 6 (1889).

Lipeurus macilhennyi Kellogg and Kuwana, *Proc. Acad. Nat. Sci. Philad.*, p. 155, pl. 7, f. 3 (1900).

Described from specimens taken off *Diomedea exulans* and by Kellogg & Kuwana (1900) from *Diomedea nigripes*. Females have been taken off *D. exulans* (Wandering Albatross) at Port Elizabeth, C.P. (1935) (coll. J. A. Pringle).

Esthiopterum stellare (Denny). See *Ardeicola stellaris* (Denny).

Esthiopterum subsignatum (Giebel). See *Anaticola subsignata* (Giebel).

Esthiopterum tricolor (Piaget).

Lipeurus tricolor Piaget, *Les Pédiculines*, p. 363, pl. 30, f. 4 (1880).

Nirmus giganticola Kellogg, *Proc. California Acad. Sci.*, VI, p. 105, pl. 5, f. 6 (1896); Bedford, *S. Afr. Ectoparasites*, p. 339 (1932).

Lipeurus confidens Kellogg, *Occ. Papers, California Acad. Sci.*, p. 26, pl. 3, f. 1 (1899).

Lipeurus miriceps Kellogg & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 480 (1902).

Lipeurus lepturus Enderlein, *Deutsch. Sud. polar Expt.*, X, p. 453 (1909).

This species has been confused with *E. obscurum* (Rudow). It has only been found on *Diomedeidae*, and has been recorded from the following South African species: *Diomedea exulans* (Wandering Albatross), *Thalassarche melanophrys* (Mollymawk), *Diomedella cauta layardi* (Layard's Albatross), *Nealbatrus chlororhynchus* (Yellow-billed Mollymawk) and *Phoebetria palpebrata* (Sooty Albatross).

Genus Ardeicola Clay.

Ardeicola Clay, *Proc. Zool. Soc. London*, iii, p. 615, t.f. 1, 3 (1935).

This genus includes species found on Gressores (storks, ibises and herons). They were formerly placed in the genus *Lipeurus* and later in *Esthiopterum*.

Genotype: Pediculus ardeae Linn.

Ardeicola ardea (Linn.).

Esthiopterum ardeae (L.) Bedford, *S. Afr. Ectoparasites*, p. 337 (1932).

Ardeicola bicolor (Piaget).

Lipeurus bicolor Piaget, *Tijd. v. Ent.*, XXXI, p. 157, pl. 4, f. 1 (1888).

Described from females and males taken off *Ephippiorhynchus* (=*Tantalus*) *senegalensis* (Saddle-bill Stork).

Ardeicola capitata (Piaget).

Esthiopterum capitatum (Piaget) Bedford, *S. Afr. Ectoparasites*, p. 337 (1932).

Ardeicola ciconia (Linn.).

Esthiopterum ciconiae (L.) Bedford, *S. Afr. Ectoparasites*, p. 338 (1932).

Ardeicola genitalis (Piaget).

Esthiopterum genitale (Piaget) Bedford, *S. Afr. Ectoparasites*, p. 339 (1932).

Ardeicola lepida (Nitzsch).

Esthiopterum lepidum (Nitzsch) Bedford, *S. Afr. Ectoparasites*, p. 339 (1932).

Ardeicola leucoprocta (Nitzsch).

Esthiopterum leucoproctum (Nitzsch) Bedford, *S. Afr. Ectoparasites*, p. 339 (1932).

Specimens have been taken off the type host, *Pyrrherodia purpurea* (Purple Heron), Worcester, C.P.

Ardeicola maculata (Nitzsch).

Lipeurus maculatus Nitzsch in Giebel, *Zeit. f. ges. Naturw.*, XXVIII, p. 383 (1866); Giebel, *Insecta Epizoa*, p. 225 (1874).

Described from specimens taken off *Ciconia nigra* (Black Stork). According to Miss Clay, it is distinct from *A. ciconia*.

Ardeicola rhaphidia (Nitzsch).

Lipeurus rhaphidius Nitzsch in Giebel, *Zeit. f. ges. Naturw.*, XXVIII, p. 384 (1866); Giebel, *Insecta Epizoa*, p. 229 (1874).

Lipeurus raphidius Piaget, *Les Pédiculines*, p. 317, pl. 26, f. 3 (1880).

Described from specimens taken off *Plegadis falcinellus* (Glossy Ibis).

Ardeicola stellaris (Denny).

Esthiopterum stellare (Denny) Bedford, *S. Afr. Ectoparasites*, p. 340 (1932).

Additional synonym: Lipeurus botauri Osborn, *Bull. Div. Ent., U.S. Dept. Agric.*, Washington, No. 5, p. 234 (1896).

Described from specimens taken off *Botaurus lentiginosus* (American Bittern).

Genus ANATICOLA Clay.

Anaticola Clay, *Proc. Zool. Soc. Lond.*, iii, p. 617, t.f. 2, 4 (1935).

This genus includes species found on Anatidae (swans, geese and ducks) and *Phoenicopteridae* (flamingoes). They were formerly placed in the genus *Lipeurus* and later in *Esthiopterum*.

Genotype: Pediculus crassicornis Scopoli.

Anaticola anseris (Linn.).

Esthiopterum anseris (Linn.) Bedford, *S. Afr. Ectoparasites*, p. 337 (1932).

Anaticola asymmetrica (Rudow).

Esthiopterum asymmetricum (Rudow) Bedford, *S. Afr. Ectoparasites*, p. 337 (1932).

Anaticola crassicornis (Scopoli).

Esthiopterum crassicorne (Scop.) Bedford, *S. Afr. Ectoparasites*, p. 338 (1932).

Anaticola subsignata (Giebel).

Esthiopterum subsignatum (Giebel) Bedford, *S. Afr. Ectoparasites*, p. 341 (1932).

Genus GIEBELIA Kellogg.

Giebelia Bedford, *S. Afr. Ectoparasites*, p. 343 (1932).

Giebelia mirabilis Kellogg.

Giebelia mirabilis Kellogg, *Proc. California Acad. Sci.*, VI, p. 138, pl. 11, f. 7, 8 (1896).

Recorded by Kellogg and Chapman (1899) taken off *Neonectris* (= *Puffinus*) *griseus* (Sooty Shearwater). It was described from specimens taken off *Puffinus opisthomelas*.

Genus CUMMINGSIELLA Ewing.

Dollabella Cummings, *Proc. Zool. Soc. Lond.*, p. 675 (1916). Bedford, *S. Afr. Ectoparasites*, p. 347 (1932).

Cummingsiella Ewing, *Proc. Biol. Soc. Wash.*, XLIII, p. 125 (1930).

As Ewing has pointed out, Cummings' name *Dollabella* is preoccupied as it was used by Giebel in 1845 for a genus of mollusks.

Genus PHILOPTERUS Nitzsch.

Philopterus Bedford, *S. Afr. Ectoparasites*, p. 348 (1932).

Philopterus albidus (Piaget).

Philopterus semisignatus (Piaget) Bedford, *S. Afr. Ectoparasites*, p. 355 (1932).

Through the kindness of Colonel R. Meinertzhagen I have been able to compare specimens of *P. semisignatus* taken off *Corvus corax corax* in the Outer Hebrides, Scotland, with specimens of *P. albidus* from *Corvus albus*, Aliwal North, C.P., and find that they are distinct species.

Philopterus gonotharax (Giebel).

Philopterus gonothorax (Giebel) Bedford, *S. Afr. Ectoparasites*, p. 351 (1932).

Docophorus lari, var. *parvus*, Piaget (*Les Pédiculines*, p. 112, 1880), described from specimens taken off *Larus dominicanus*, proves to be a synonym of this species.

Philopterus snyderi (Kellogg & Paine).

Docophorus snyderi Kellogg & Paine, *Ent. News*, XXI, p. 124, f. 1, 2 (1910).

Philopterus snyderi (Kellogg & Paine) Ferris, *Bern. P. Bishop Mus. Honolulu*, Bull. 98, pp. 71-72, t.f. 20A-D (1932).

Specimens taken off *Sterna fuliginosa*, Eshowe, Zululand (coll. B. De Meillon). This species was described from specimens taken off *Sterna lunata*, Laysan Is.; also recorded from *Sterna fuscata*, Marquesas.

Family TRICHODECTIDAE.

Genus DASYONYX Bedford.

Dasyonyx Bedford, *S. Afr. Ectoparasites*, p. 359 (1932).

Dasyonyx windhuki Bedford.

Dasyonyx windhuki Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 38, f. 6-7, (1936).

Described from males and females taken off *Procavia windhuki*, Naukluft, South-West Africa; also from *Procavia* sp. Otjosongomha, Waterberg, South-West Africa.

Dasyonyx validus Bedford.

Trichodectes lindfieldi Ferris, *Rep. Harvard Afr. Exped. upon Afr. Repub. Liberia and Belg. Congo*, ii, p. 1030, f. 19, 20A, C, D, E, G, H (1930), *nec*. Hill, 1928.

Dasyonyx validus Bedford, *Proc. Zool. Soc. London*, p. 721 (1932).

Males from *Dendrohyrax arborea*, Port Alfred, C.P. (coll. R. F. Lawrence). Also recorded by Ferris (1930) from *D. adolfi-friederici*, Belgian Congo, and *D. validus*, Mt. Kilimanjaro, and by Bedford (1936) from *D. scheelei*, Tanganyika Territory.

Genus PROCAVIPHILUS Bedford.

Procaviphilus serraticus (Hill).

Procaviphilus serraticus (Hill) Bedford, *S. Afr. Ectoparasites*, p. 361 (1932).

Additional record: One female from *Procavia windhuki*, Naukluft, South-West Africa (coll. K. Jordan).

Genus BOVICOLA Ewing.

Bovicola Bedford, *S. Afr. Ectoparasites*, p. 361 (1932).

Bovicola hilli Bedford.

Bovicola hilli Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, II, i, p. 43, t.f. 4-6 (1934).

Described from specimens taken off Waterbuck, *Kobus ellipsiprymnus* (Ogilby), Umfolosi Game Reserve, Zululand. It has also been found on *Kobus defassus*, Uganda.

Bovicola crassipes (Rudow).

Bovicola limbatus (Gervais) Bedford, *S. Afr. Ectoparasites*, p. 362 (1932).

According to Bedford (1936) Gervais' *Trichodectes climax* and *T. limbatus* should be discarded; *Trichodectes pilosus* Piaget (*Les Pédiculines*, p. 406, pl. 48, f. 4, 1880), *nec* Giebel, 1874, should be sunk as a synonym of this species, and *T. climax*, var. *major*, Piaget is the same as *B. painei* (Kellogg & Nakayama).

Bovicola major (Piaget).

Trichodectes climax, var. *major*, Piaget, *Les Pédiculines*, Suppl., p. 86, pl. 9, f. 5 (1885).

Trichodectes painei Kellogg & Nakayama, *Psyche*, XXI, p. 90, f. 1 (1914).

Bovicola painei (K. & N.) Bedford, *S. Afr. Ectoparasites*, p. 362 (1932).

B. major has been confused with *B. limbatus*. It proves to be the same as *B. painei* over which it has priority (see Bedford, 1936).

Bovicola martinaglia Bedford.

Bovicola martinaglia Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 43, f. 11-13 (1936).

Described from specimens taken off *Onotragus leche* in the Zoological Garden, Johannesburg.

Bovicola painei (Kellogg & Nakayama). See *Bovicola major* (Piaget).

Bovicola pelea Bedford.

Bovicola pelea Bedford, *Onderstepoort Jour., Vet. Sci. & Anim. Indust.*, II, i, p. 41, t.f. 1-3 (1934).

Described from specimens taken off Vaal Rhebok, *Pelea capreolus* (Behst.), Naauwpoort, C.P.

Genus TRICHOLIPEURUS Bedford.

Tricholipeurus aepycerus Bedford.

Trichlipeurus aepycerus Bedford, *S. Afr. Ectoparasites*, p. 363 (1932).

Described from specimens reported to have been taken off an Impala, *Aepyceros melampus*, on the Kunene River, South-West Africa, but Bedford (1934) has shown that the host was probably *Aepyceros petersi* Boch.

Tricholipeurus elongatus Bedford.

Tricholipeurus elongatus Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, II, i, p. 46, t.f. 7-9 (1934).

Described from specimens taken off an Impala, *Aepyceros melampus*, Kruger National Park, Transvaal.

Genus DAMALINIA Mjöberg.

Damalinia Bedford, *S. Afr. Ectoparasites*, p. 364 (1932).

Damalinia hopkinsi Bedford.

Damalinia hopkinsi Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 47, f. 18-19 (1936).

This species, described from specimens taken off Eland (*Taurotragus oryx pattersonianus*) in Uganda, is certain to be found also on the South African Eland.

Genus FELICOLA Ewing.

Felicola Bedford, *S. Afr. Ectoparasites*, p. 365 (1932).
Suricatoecus Bedford, *ibid.*, p. 365 (1932).

Suricatoecus proves to be a synonym of *Felicola.*

Felicola acuticeps (Neumann).

Trichodectes acuticeps Neumann, *Archiv. Parasit.*, V, p. 601 (1902).

Felicola genetta (Bedford) Bedford, *S. Afr. Ectoparasites*, p. 366 (1932).

Trichodectes genetta Bedford proves to be a synonym of *T. acuticeps* Neu. I am deeply indebted to Mr. Fabio L. Werneck for very kindly sending me photographs of drawings he made of the types of *T. acuticeps.*

Felicola cooleyi (Bedford).

Suricatoecus cooleyi Bedford, *S. Afr. Ectoparasites*, p. 365 (1932).

Additional record: Specimens have been taken off *Mungos mungo colonus*, Uganda.

Superfamily AMBLYCERA.

Family MENOPONIDAE.

Genus NUMIDICOLA Ewing.

Numidicola longicornis Ewing, the type of the genus, is undoubtedly a synonym of *N. antennata* (Kellogg & Paine).

Numidicola antennata (Kellogg & Paine).

Numidicola antennata (Kellogg & Paine) Bedford, *S. Afr. Ectoparasites*, p. 371 (1932).

Additional record: Specimens taken off *Numida coronata*, Vryburg, C.P.

Genus MENOPON Nitzsch.

Menopon Bedford, *S. Afr. Ectoparasites*, p. 372 (1932).

Menopon inaequale Piaget. See *Myrsidea inaequalis* (Piaget).

Menopon paululum Kellogg & Chapman.

Menopon paululum Kellogg & Chapman, *Occ. Papers, California Acad. Sci.*, VI, p. 119, pl. 8, f. 2 (1899).

Described from specimens taken off *Puffinus opisthomelas*, *P. creatopus* and *Neonectris* (= *Puffinus*) *griseus* (Sooty Shearwater), the last-named occurring on the South African coast.

Menopon petulans Kellogg & Chapman.

Menopon petulans Kellogg & Chapman, *Occ. Papers, California Acad. Sci.*, VI, p. 121, pl. 8, f. 3 (1899).

Described from a male taken off *Neonectris* (= *Puffinus*) *griseus* (Sooty Shearwater), Bay of Monterey, California.

Genus UCHIDA Ewing.

Neumannia Uchida, *Jour. Coll. Agr. Imp. Univ. Tokyo*, IX, i, p. 27 (1926), *nec* Trouessart (1888); Bedford, *S. Afr. Ectoparasites*, p. 378 (1932).

Uchida Ewing, *Proc. Biol. Soc. Washington*, XLIII, p. 125 (1930).

As Ewing has pointed out, Uchida's name *Neumannia* cannot stand as it was used by Trouessart in 1888 for a genus of Acarina.

Genus MENACANTHUS Neumann.

Menacanthus Bedford, *S. Afr. Ectoparasites*, p. 378 (1932).

Menacanthus gonophaeus (Burmeister).

Menopon gonophaeum Burmeister, *Handbuch der Ent.*, II, p. 440 (1838); Giebel, *Insecta Epizoa*, p. 282, p. 15, f. 1 (1874).

Menopon ovatum Piaget, *Les Pédiculines*, p. 430, pl. 34, f. 6 (1880).

Myrsidea gonophaea (Nitzsch) Harrison, *Parasit.*, IX, i, p. 58 (1916).

Myrsidea ovata (Piaget) Harrison, *ibid*, IX, p. 59 (1916); Bedford, *S. Afr. Ectoparasites*, p. 382 (1932).

Menacanthus corvus Bedford, *Rep. Dir. Vet. Serv. & Anim. Indust., Un. S. Afr.*, XVI, p. 155, f. 1-3 (1930); Bedford, *S. Afr. Ectoparasites*, p. 379 (1932).

The types of *gonophaeus* were taken off *Corvus corax* in Europe, and those of *ovatus* and *corvus* off *Corvus albus* (= *C. scapulatus*), and the latter also from *Heterocorax capensis*. Through the kindness of Mr. C. D. Radford I have received specimens taken off *Corvus frugilegus* in England.

Harrison (1916) sank *Menopon anaspilum* Nitzsch as a synonym of *Menopon gonophaeum* Nitzsch, but I have received through the kidness of Colonel R. Meinertzhagen specimens taken off *Corvus corax corax* (type host) in the Outer Hebrides, Scotland, and find that it is a distinct species of *Myrsidae*.

Genus MYRSIDEA Waterston.

Myrsidea Bedford, *S. Afr. Ectoparasites*, p. 382 (1932).

Myrsidea inaequalis (Piaget).

Menopon inaequale Piaget, *Les Pédiculines*, p. 443, pl. 35, f. 1 (1880).

Menopon inaequale (Piaget) Bedford, *S. Afr. Ectoparasites*, p. 373 (1932).

Described from specimens taken off Red-backed Shrike, *Enneoctonus collurio* (= *Lanius collurio*), a migrant to South Africa. It is probably a synonym of *Menopon coarctatum* (Scopoli).

Myrsidea ovata (Piaget). See *Menacanthus gonophaeus* (Nitzsch).

Genus TRINOTON Nitzsch.

Trinoton aculeatum Piaget.

Trinoton aculeatum (Piaget) Bedford, *S. Afr. Ectoparasites*, p. 382 (1932).

Additional record: Specimens taken off *Paecilonitta erythrorhyncha* (Red-billed Teal), Rustenburg District, Transvaal.

Genus HELEONOMUS Ferris.

Heleonomus miandrius (Kellogg).

Heleonomus miandrius (Kellogg) Bedford, *S. Afr. Ectoparasites*, p. 385 (1932).

Heleonomus confusus (Ferris) Bedford, *ibid.*, p. 384 (1932).

Described by Kellogg (1910) from specimens taken off *Balearica regulorum gibbericeps* in East Africa. Later Ferris (1916) considered that the female described by Kellogg was distinct from the male, and proposed the name *confusus* for it. Kellogg in describing the female states that it is markedly smaller than the male, and has a narrow uncoloured median longitudinal line extending from abodominal segment 3 along the thoracic segments, and to the middle of the head, where it forks. Both the size and uncoloured line indicate that the specimens were immature; similar lines being found in immature specimens of species of *Actornithophilus*, *Trinoton*, etc. I have examined males, females and immature females sent me by Dr. H. Schouteden taken off *Balearica regulorum* (Crowned Crane) in the Belgian Congo, and find that the immature specimens agree perfectly with Kellogg's description, except that the line extends to the fourth abdominal segment. The mature females are slightly larger than the males, and the plates on tergites i-iv are divided in the middle by a narrow line as in immature specimens, except the fourth, which is only partly divided.

Genus COLPOCEPHALUM Nitzsch.

Colpocephalum Bedford, *S. Afr. Ectoparasites*, p. 388 (1932).

Colpocephalum angulaticeps Piaget.

Colpocephalum angulaticeps Piaget, *Les Pédiculines*, p. 569, pl. 47, f. 8 (1880).

Colpocephalum spineum Kellogg, *Occ. Papers, California Acad. Sci.*, VI, pp. 38-39, pl. 4, f. 1 (1889); Kellogg & Kuwana, *Proc. Wash. Acad. Sci.*, IV, p. 484 (1902); Waterston, *Nat. Hist. Rep. Brit. Antarctic Exped. Zool.*, III, p. 270 (1921).

Colpocephalum angulaticeps (Piaget) Ferris, *Bern. P. Bishop. Mus., Honolulu*, Bull. 98, p. 54, f. 8A-F (1932); Bedford, *S. Afr. Ectoparasites*, p. 389 (1932).

Ferris has shown *spineum* to be a synonym of *angulaticeps*. The former was described by Kellogg from *Fregata aquila*, Panama, and the latter from *Fregata minor*. It has also been recorded from other sea-birds, but its presence on these was in all probability due to straggling, except possibly, as Ferris has stated, its occurrence on *Sula websteri*.

Colpocephalum decimfasciatum Boisduval & Lacordavei.

Colpocephalum decimfasciatum Bois. & Lac. Bedford, *S. Afr. Ectoparasits*, p. 380 (1932).

Additional synonym: Colpocephalum laticeps Kellogg, *Proc. Calif. Acad. Sci.*, VI, p. 149, pl. 12, f. 8 (1896). Described from specimens taken off *Ardea egretta,* Kansas, U.S.A., and *Botaurus lentiginosus*, Colorado, U.S.A.

Colpocephalum nyctarde Denny from *Nycticorax nyctocorax* is probably also a synonym.

Additional records: From Buff-backed Egret, *Bubulcus ibis* (L.), Johannesburg (coll. G. Martinaglia), Rustenburg District (coll. W. Powell), and Sandford, Bergville District (coll. J. R. Frean). Specimens have also been received from Mr. L. H. Dunn taken off *Nycticorax nycticorax naevius* (Boddaert), Panama, and from Mr. H. S. Peters taken off *Ardea herodius herodius* (Great Blue Heron), Fredericktown, Ohio, U.S.A. (coll. L. E. Hicks).

Colpocephalum ephippiorhynchi Mjöberg. See *C. zebra* Nitzsch.

Colpocephalum oreas Kellogg. See *C. zebra* Nitzsch.

Colpocephalum semicinctum Rudow. See *C. subaequale* Nitzsch.

Colpocephalum subaequale Nitzsch.

Colpocephalum subaequale Nitzsch in Burmeister, *Handbuch*, II, p. 438 (1838); Giebel, *Insecta Epizoa*, p. 265, pl. 13, f. 13-14 (1874); Kellogg, *Proc. California Acad. Sci.*, VI, p. 525, pl. 72, f. 1 (1896).

Colpocephalum semicinctum Rudow, *Zeit. f. ges. Naturw.*, XXVII, p. 471 (1866); Bedford, *S. Afr. Ectoparasites*, p. 392 (1932).

The types of *subaequale* were taken off *Corvus corax* and *C. frugilegus* in Europe, and the type of *semicinctum* off *Corvus albus* (= *C. scapulatus*). Through the kindness of Colonel R. Meinertzhagen and Mr. C. D. Radford, I have received specimens taken off *Corvus corax, Corvus frugilegus* and *Pyrrhocorax pyrrhocorax* in England, and find they are identical with specimens taken off *Corvus albus.*

Specimens have been taken off *Corvultur albicollis* (White-necked Raven), Maseru, Basutoland, and Maziba, Uganda (coll. G. H. E. Hopkins) Kellogg (1896 and 1899) has recorded it from the American crows, *Corvus americanus* and *C. corax sinuatus.*

Colpocephalum zebra Nitzsch.

Colpocephalum zebra Nitzsch, in Burmeister, *Handbuch*, II, p. 438 (1838); Denny, *Anoplur. Brit.*, p. 210, pl. 19, f. 2 (1842); Piaget, *Les Pédiculines*, pp. 545-546, pl. 45, f. 5 (1880).

Colpocephalum ephippiorhynchi Mjöberg, *Arkiv. f. Zool.*, VI, p. 43, pl. 3, f. 9 (1910).

Colpocephalum oreas Kellogg, *Schwed. Exp. Kilimanjaro*, p. 51, pl. 7, f. 8 (1910).

Colpocephalum zebra (Nitzsch) Ferris, *Parasit.*, XVI, i, p. 59, f. 2A-G (1924); Bedford, *S. Afr. Ectoparasites*, p. 394 (1932).

Specimens recently taken off *Ephippiorhynchus senegalensis* (Saddle-bill Stork), Mazabuka, Northern Rhodesia (coll. P. L. le Roux), prove that both *C. ephippiorhynchi* and *C. oreas*, which were described from specimens taken off the same species of host, are synonyms of *C. zebra*.

Suborder SIPHUNCULATA.

Family HAEMATOPINIDAE Enderlein.

Genus SCIPIO Cummings.

Scipio tripedatus Ferris.

Scipio tripedatus Ferris, *Contrib. Toward Mon. Suck. Lice*, Part V, p. 15 (285), t.f. 173-175 (1932).

Scipio nov. sp. Bedford, *S. Afr. Ectoparasites*, p. 402 (1932).

Described from a female and immature specimens taken off a "nokey" in South Africa; also a male from *Petromys typicus tropicalis*, Windhoek, South-West Africa, and specimens from *P. typicus*, Soebatsfontein, near Garies, Namaqualand (coll. R. F. Lawrence).

Genus POLYPLAX Enderlein.

Polyplax Bedford, *S. Afr. Ectoparasites*, p. 403 (1932).

Polyplax serrata (Burmeister).

Pediculus serratus Burmeister, "*Rhynchota*", *Gen. Ins.*, No. 6 (1839).

Polyplax serrata (Burm.) Ferris, *Contrib. Towards Mon. Suck. Lice*, iv, pp. 191-192, t.f. 120B-E (1923).

Specimens have been taken off a White Mouse at Onderstepoort (coll. G.A.H.B.). It was described from specimens taken off *Mus musculus* (House Mouse) in Europe.

Polyplax spinulosa (Burmeister).

Polyplax spinulosa (Burm.) Bedford, *S. Afr. Ectoparasites*, p. 405 (1932).

Additional record: Grammomys dolichurus (Cape Arboreal Rat), Pietermaritzburg, Natal (coll. V. F. Woodiwiss).

Genus *Hoplopleura* Enderlein.

Hoplopleura biseriata Ferris

Hoplopleura biseriata (Ferris) Bedford, *S. Afr. Ectoparasites*, p. 406 (1935).

Additional record: Specimens received from Dr. B. De Meillon taken off *Tatera joanae* Thos., Ondongo, South-West Africa (coll. K. Schetter).

Genus HAEMODIPSUS Enderlein.

Haemodipsus Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 59 [329] (1932).

Haemodipsus Bedford, *S. Afr. Ectoparasites*, p. 407 (1932).

This genus now comprises four species found on Leporidae.

Haemodipsus africanus Bedford.

Haemodipsus africanus Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, II, i, p. 48, t.f. 10 (1934).

Described from a female taken off *Lepus zuluensis* Thos. & Schw., Jericho, Transvaal.

Genus PROLINOGNATHUS Ewing.

Prolinognathus Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 138 [408] (1932).

Prolinognathus Bedford, *S. Afr. Ectoparasites*, p. 408 (1932).

This genus includes two species found on Procaviidae, both of which may occur on the same host.

Prolinagnathus caviae-capensis (Cummings).

Prolinognathus caviae-capensis (Cumms.) Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 139 [409], t.f. 250A, 251A, E-J (1932).

Prolinognathus caviae-capensis (Pallas) Bedford, *S. Afr. Ectoparasites*, p. 408 (1932), part.

As Ferris (1932) has pointed out, this species should be credited to Cummings (1913) rather than to Pallas (1767), and has been confused with *P. leptocephalus* (Ehrenberg). Both Pallas and Cummings recorded it from *Procavia capensis*, and specimens have been taken off the same host species at Hout Bay, near Capetown; also off *Procavia coombsi*, Onderstepoort. Specimens recorded from other hosts prove to be *P. leptocephalus*.

Prolinognathus leptocephalus (Ehrenberg).

Pediculus leptocephalus Ehrenberg, *Symbolae Physicae, Decas Prima*, p. f. (1828).

Prolinognathus leptocephalus (Ehrenb.) Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 142 [412], t.f. 250B, 251B-D (1932).

Originally described from *Procavia* (= *Hyrax*) *syriacus*, and Ferris has recorded it from the same host species from Syria. Specimens have also been taken off *Procavia coombsi*, Onderstepoort and Rooikrans, Transvaal; *Procavia capensis*, Hout Bay, near Capetown (coll. R. F. Lawrence); *Procavia* sp., Lamberts Bay, C.P. (Tvl. Mus. No. 2148); *Procavia waterbergensis*, Otjiwarongo, South-West Africa (Tvl. Mus. No. 5335); *Heterohyrax ruddi*, Macequece, Portuguese East Africa (Tvl. Mus. No. 6215), and Ferris (1932) has recorded it from *Procavia brucei rudolfi*, Marsabit Road, Kenya Colony.

Genus LINOGNATHUS Enderlein.

Linognathus Ferris, *Contrib. Toward Mon. Sucking Lice*, V, p. 66 [336] (1932).

Linognathus Bedford, *S. Afr. Ectoparasites*, p. 408 (1932).

This genus has recently been monographed by Ferris and now comprises twenty-seven species found on Bovidae and domestic dog; of these twenty have been recorded from South Africa

Linognathus aepycerus Bedford.

Linognathus aepycerus Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 62, t.f. 5-6 (1936).

Described from males and females taken off *Aepyceros melampus* (Impala), between Pretoria and Johannesburg; also females off the same host, Rustenburg District, Transvaal.

Linognathus angulatus (Piaget). See *Linognathus gazella* Mjöberg.

Linognathus bedfordi Ferris.

Linognathus bedfordi Ferris, *Contrib. Toward. Mon. Suck. Lice*, V, p. 117 [387], t.f. 236, 238B, C, E, H, 239A-D (1932).

Described from specimens taken off *Antidorcas marsupialis* (Springbok) at Onderstepoort.

Linognathus damaliscus Bedford.

Linognathus damaliscus Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 61, t.f. 3-4 (1936).

Described from males and females taken off *Damaliscus albifrons* (Blesbok). Zoological Gardens, Johannesburg, and females off *Damaliscus dorcas* (Bontebok), Bredasdorp, C.P.

Linognathus fractus Ferris.

Linognathus fractus Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 96 [366], t.f. 220, 221 (1932).

Linognathus nov. sp. Bedford, *S. Afr. Ectoparasites*, p. 409 (1932).

Described from specimens taken off *Tragelaphus sylvaticus* (Bushbuck) at Onderstepoort.

Linognathus gazella Mjöberg.

Linognathus gazella Mjöberg, *Arkiv. för Zoologi*, VI, p. 157, f. 78 (1910).

Linognathus gilvus Fahrenholz, *Jahrbuch der Hamburg Wissensch. Anstal.*, XXXIV, p. 18, f. 5 (1917).

Linognathus gazella (Mjöberg) Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 111 [381], t.f. 232B, E, F, 234 (1932).

Linognathus angulatus Bedford, *S. Afr. Ectoparasites*, p. 409 (1932).

As Ferris (1932) has pointed out, specimens recorded by Ferris (1916) and Bedford (1927 and 1932) taken off *Cephalopus natalensis* (Red Duiker), Mfongosi, Zululand and *Sylvicapra grimmi* (Duiker) from Transvaal and Zululand prove to be *L. gazella*. Bedford (1932) also recorded it as *L. angulatus* from *Philantomba monticola* (Blue Duiker), Pietermaritzburg, Natal. *L. gazella* was described by Mjöberg from "gazella", Hamburg Zoological Gardens, and also erroneously recorded by him as *L. angulatus* from *Cephalophus* sp. in the same gardens. The latter were later described by Fahrenholz as *L. gilvus*.

L. angulatus is only known from *Cephalopus nigrifrons* and should be deleted from the South African list.

Linognathus hippotragi Ferris.

Linognathus hippotragi Ferris, *Contrib. Toward Mon. Suck. Lice*, V, p. 103 [373], t.f. 226, 227 (1932).

Described from specimens taken off *Hippotragus niger* (Sable Antelope) in the Zoological Gardens, Johannesburg.

Linognathus limnotragi Cummings.

Linognathus limnotragi Cummings, *Bull. Ent. Res.*, IV, p. 36, f. 1 (1913).

Linognathus limnotragi (Cumms.) Ferris, *Contrib. Toward Mon. Suck. Lice*, V. p. 113 [383], t.f. 231A, I-J, 232C-D (1932).

Recorded by Ferris taken off *Tragelaphus sylvaticus* (Bushbuck) at Onderstepoort; also from *Tragelaphus scriptus*, Zoological Gardens, London. It was described by Cummings from specimens taken off *Limnotragus* (=*Tragelaphus*) *gratus*, in the Zoological Gardens, London, from the Congo.

Linognathus peleus Bedford.

Linognathus peleus Bedford, *Onderstepoort Jour. Vet. Sci. & Anim. Indust.*, VII, i, p. 59, t.f. 1-2 (1936).

Described from males and females taken off *Pelea capreolus* (Vaal Rhebok),. Naauwpoort, C.P.

Linognathus spicatus Ferris.

Linoganthus spicatus Ferris, *Contrib. Toward Mon. Suck Lice*, V, p. 118 [388], t.f. 237, 238A, G, 239E (1932).

Described from specimens taken off *Gorgon taurinus* (Blue Wildebeest), Maasstroom, Northern Transvaal. Specimens have also been taken off the same host species in the Zoological Gardens, Pretoria.

Genus HAEMATOPINUS Leach.

Haematopinus Bedford, *S. Afr. Ectoparasites*, p. 411 (1932).

Haematopinus Ferris, *Contrib. Toward Mon. Suck. Lice*, VI, p. 5 [419] (1933).

This genus has recently been monographed by Ferris and now comprises eleven species found on ungulates; of these eight are parasitic on South African hosts.

Haematopinus acuticeps Ferris.

Haematopinus acuticeps Ferris, *Contrib. Toward Mon. Suck. Lice*, VI, p. 53 [467], f. 275 (1933).

Described from females taken off *Hippotigris burchelli* (Zebra), Mpwapwa, Tanganyika Territory.

Genus PHTHIRUS Leach.

Phthirus pubis (Linnaeus).

Phthirus pubis (Linn.) Bedford, *S. Afr. Ectoparasites*, p. 414 (1932).

Specimens have been received from Dr. R. F. Lawrence taken off a chimpanzee in the French Congo along with specimens of *Pediculus schaäffi* Fahrenholz.

Order HEMIPTERA (=RHYNCHOTA).

Suborder HETEROPTERA.

Genus CACODMUS Stal.

Cacodmus sparsilis Rothschild.

Cacodmus sparcilis (Roths.) Bedford, *S. Afr. Ectoparasites*, p. 416 (1932).

Specimens have been taken off *Scotophilus nigrita dingani* at Onderstepoort. Previously recorded taken from the same host in Natal.

Cacodmus villosus (Stal).

Cacodmus villosus (Stal) Bedford, *S. Afr. Ectoparasites*, p. 415 (1932).

Additional records: From *Rousettus collaris* (Illiger), Pietermaritzburg, Natal, and *Eptesicus capensis*, Onderstepoort (coll. G.A.H.B.).

Genus APHRANIA Jordan & Rothschild.

Aphrania Jordan & Roths., *Novit. Zool.*, XIX, p. 353 (1912).

Generic characters: Setae shorter than in *Cacodmus*, only a small number of lateral ones on the pronotum longer than the first antennal segment. Second segment of proboscis shorter than fourth. Hind tibia with distinct pseudo-joint, mid tibia with indistinct joint, and fore tibia without pseudo-joint. This genus contains a single species.

Aphrania barys Jordan & Rothschild.

♂ *Aphrania barys* Rothschild & Jordan, *Novit. Zool.*, XIX, p. 355-356, t.f. 3, 4 (1912).

Described from males collected at Maseru, Basutoland. The host is unknown.

Order DIPTERA.

DIPTERA PUPIPARA.

Family HIPPOBOSCIDAE.

Genus HIPPOBOSCA Linn.

Hippobosca Bedford, *S. Afr. Ectoparasites*, p. 418 (1932).

Hippobosca martinaglia Bedford.

Hippobosca martinaglia Bedford, *Onderstepoort Journ. Vet. Sci. & Anim. Indust.*, VII, i, p. 67, f. 1 (1936).

Described from females and a male taken off Impala, *Aepyceros melampus* (Licht.), Bar R Ranch, Swaziland.

Genus ORNITHEZA Speiser.

Ornitheza metallica (Schiner).

Ornitheza metallica (Sch.) Bedford, *S. Afr. Ectoparasites*, p. 421 (1932).

Additional record: One female from Layard's *Bulbul, Loidorusa layardi* (Gurn.), Onderstepoort, 2 Nov., 1933 (coll. G.A.H.B.).

Genus CRATAERINA von Olfers.

Crataerina Bedford, *S. Afr. Ectoparasites*, p. 425 (1932).

Crataerina melbae (Rondani)..

Chelidomyia melbae Rondani, *Bull. Soc. Ent. Ital.*, XI, p. 17 (1879).

Crataerina melbae (Rond.) Austen, *Parasit.*, XVIII, iii, pp. 358-359 (1926).

One male and one female taken off African Great Swift, *Tachymarptis melba africana* (Temm.), Mamathes, Basutoland, 12th December, 1933 (coll. C. J. Guillarmod). In the female the second basal cell is divided into two by a supernumerary transverse vein, but in the male this cell is not divided. Normally it is divided in both sexes.

This species has previously been reported taken off the Alpine Swift, *T. melba* (L.). Austen records one male from Switzerland and one female without locality label.

Family STREBLIDAE.

Genus RAYMONDIA Frauenfeld.

Raymondia bedfordi Ferris.

Raymondia bedfordi (Ferris) Bedford, *S. Afr. Ectoparasit.*, p. 427 (1932).

Additional record: From *Petalia damarensis* (Ptrs.), Gokha Hills.

Raymondia huberi huberi Frauenfeld.

Raymondia huberi (Frauenfeld) Bedford, *S. Afr. Ectoparasites*, p. 427 (1932).

Additional records: From *Hipposideros caffer* (Sund.), Tzaneen, Northern Transvaal (coll. H. H. Dilly), and *Cleotis percivalli australis* Rbts., Uitkyk, near Krugersdorp, Transvaal (coll. G. van Son).

Raymondia waterstoni Jobling.

♀ ♂ *Raymondia waterstoni* Jobling, *Parasit.*, XXIII, i, pp. 79-83, t.f. 1, 2A-D (1931).

Described from specimens taken off *Rhinolophus simulator* from Tanganyika Territory.

Additional records: From *Rhinolophus geoffroyi augur* Anders., in a cave, Pretoria (coll. G.A.H.B.); also from *Rhinolophus empusa* and *Clocotis percivalli australis* Rbts., Uitkyk, near Krugersdorp, Transvaal (coll. G. van Son).

Genus NYCTERIBOSCA Speiser.

Nycteribosca Jobling, *Parasit.*, XXVI, i, pp. 64-69 (1934).

This genus now contains 17 species, one of which is doubtful. Of these, only two have been recorded from the Ethiopian region.

Nycteribosca africana (Walker).

Strebla africana Walker, *List of the Specimens of Diptera Insects in the Collection of the British Museum*. Part IV, 1146 (1849).

Raymondia kollari Frauenfeld, *Sitzunsber*, *Akad. Wiss. Wien*, XVIII, p. 329, f. 1A-B (1855), *nec* Schiner, 1868.

Nycteribosca kollari Speiser, *Arch. f. Naturg.*, LXVI, p....... (1900).

Nycteribosca africana (Walker) Jobling, *Parasit.*, XXVI, i, pp. 86-88, t.f. 10A-C (1934).

Nycteribosca africana (Walker) Bedford, *S. Afr. Ectoparasites*, p. 427 (1932).

Nycteribosca kollari (Speiser) Bedford, *ibid.*, p. 427 (1932).

As Jobling has shown, *Nycteribosca kollari* (Frauenfeld) is a synoym of *Nycteribosca africana* (Walker).

Additional record: From *Rhinolophus empusa*, Uitkyk, near Krugersdorp, Transvaal (coll. G. van Son).

Order SIPHONAPTERA.

Family TUNGIDAE.

Genus ECHIDNOPHAGA Olliff.

Echidnophaga larina Jordan & Rothschild.

Echidnophaga larina (Jord. & Roths.) Bedford, *S. Afr. Ectoparasites*, p. 435 (1932).

Additional record: Domestic Pigs, Pretoria District.

Family PULICIDAE.

Genus PROCAVIOPSYLLA Jordan.

Procaviopsylla angolensis Jordan.

Procaviopsylla angolensis (Jordan) Bedford, *S. Afr. Ectoparasites*, p. 438 (1932).

Additional record: Specimens have been collected by Dr. B. De Meillon from *Heterohyrax granti*, Zoutpansberg, Northern Transvaal.

Genus XENOPSYLLA Glinkiewiez.

Xenopsylla Bedford, *S. Afr. Ectoparasites*, p. 440 (1932).

Xenopsylla cheopis (Rothschild).

Xenopsylla cheopis (Roths.) Bedford, *S. Afr. Ectoparasites*, p. 442 (1932).

Additional record: Mus musculus (House Mouse), Pietermaritzburg, Natal (coll. V. F. Woodiwiss).

Xenopsylla phyllomae De Meillon.

♂ *Xenopsylla phyllomae* De Meillon, *Publ. S. Afr. Instit. Med. Res.* (33), VI, p. 265, pl. 4, f. 1-2 (1934).

Described from a male taken off *Aethomys chrysophilus tzaneenensis* (Jameson), Zoutpansberg, Northern Transvaal.

Genus CTENOCEPHALIDES Stiles & Collins.

Ctenocephalides connatus (Jordan).

Ctenocephalides connatus (Jordan) Bedford, *S. Afr. Ectoparasites*, p. 447 (1932).

Additional record: Specimens taken off a hare, Tzaneen, Northern Transvaal (coll. B. De Meillon).

Genus CERATOPHYLLUS Curtis.

Ceratophyllus Bedford, *S. Afr. Ectoparasites*, p. 448 (1932).

Jordan (1933) has recently split this genus up into a number of genera. Both *C. fasciatus* (Bosc.) and *C. londiniensis* Rothschild are now included in the genus *Nosopsyllus* Jordan.

Genus NOSOPSYLLUS Jordan.

Nosopsyllus Jordan, *Novit. Zool.*, XXXIX, i, p. 76 (1933).

Genotype: Ceratophyllus fasciatus (Bosc.).

(See note under previous genus.)

Genus CHIASTOPSYLLA Rothschild.

Chiastopsylla rossi (Waterston).

Chiastopsylla rossi (Waterst.) Bedford, *S. Afr. Ectoparasites*, p. 453 (1932).

Additional record: Specimens taken off *Grammomys dolichurus* (Cape Arboreal Rat), Pietermaritzburg, Natal (coll. V. F. Woodiwiss).

Genus CTENOPHTHALMUS Kolenati.

Ctenophthalmus calceatus Waterston.

Ctenophthalmus calceatus (Waterst.) Bedford, *S. Afr. Ectoparasites*, p. 457 (1932).

Additional records: Specimens have been received from Dr. V. F. Woodiwiss taken off the following hosts at Pietermaritzburg, Natal: *Otomys irroratus* (Water Rat), *Mus musculus* (House Mouse), *Rhabdomys pumilio* (Striped Mouse), *Rattus rattus* (Black Rat) and *Grammomys dolichurus* (Cape Arboreal Rat).

Genus DINOPSYLLUS Jordan & Rothschild.

Dinopsyllus lypusus Jordan & Rothschild.

Dinopsyllus lypusus J. & R. Bedford, *S. Afr. Ectoparasites*, p. 458 (1932).

Additional records: Specimens have been taken off *Mus musculus* (House Mouse) and *Grammomys dolichurus* (Cape Arboreal Rat), Pietermaritzburg, Natal (coll. V. F. Woodiwiss).

Genus LEPTOPSYLLA Jordan and Rothschild.

Leptopsylla segnis (Schönh.).

Leptosylla segnis (Schönh) Bedford, *S. Afr. Ectoparasites*, p. 460 (1932).

Additional record: Specimens taken off *Grammomys dolichurus* (Cape Arboreal Rat), Pietermaritzburg, Natal (coll. V. F. Woodiwiss).

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Section III.

Bacteriology.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Pasteurellosis: An Outbreak Amongst Sheep.

By M. W. HENNING AND M. H. V. BROWN, Sections of Bacteriology and Pathology, Onderstepoort.

INTRODUCTION AND BRIEF SUMMARY OF LITERATURE.

CASES of pasteurellosis or infection with bipolar organisms in domestic animals and birds are frequently reported from different parts of South Africa; but, apart from the description of the disease in sheep given by Maybin (1931) and the account of Fowl Cholera by Henning and Coles (1933), the information furnished regarding the incidence of pasteurellosis in this country is unreliable and incomplete. According to some of the routine pathological specimens received at Onderstepoort, however, it would appear that pasteurellosis in sheep is far more widespread than is generally recognised. Maybin found the disease extremely prevalent in South-West Africa, where it affected goats as well as various breeds of sheep, the highest mortality occurring in Karakul sheep and their crosses; from the pathological specimens received at Onderstepoort it appears that in the Union Persian sheep are mostly affected, Merino sheep not having made any significant contribution.

In Europe haemorrhagic septicaemia of sheep was first described in 1889 (Galtier) and since then it has been recognised over wide areas of the continent. The relation, however, between European haemorrhagic septicaemia of sheep and the condition here described is not clear. Hutyra and Marek (1926) describe peracute, acute, subacute and chronic forms of the disease whereas in this outbreak these forms could not be differentiated—apart from one or two acute cases in the experimental animals only the chronic form with the development of pneumonia and pleuritis was seen.

Leyshon's (1932) description of "An Ovine Affection" coincides more closely with the condition recorded here. He observed cases of *pasteurella* infection in sheep where the animals lived from twelve hours to three or four days, and showed at autopsy pneumonia with numerous bipolar organisms present. His description agrees largely with Dungall's (1931) account of a contagious pneumonia of sheep occurring in Iceland between October and June; from some of these cases bipolar organisms pathogenic to sheep were isolated. Laikipia lung disease (Mettam, 1930) appears to be another form of this condition. Curasson and Didier (1932) record heavy mortality in sheep, especially amongst young animals, due to a *pasteurella* infection. It is interesting to note that these workers used a vaccine made from a formolised culture which was claimed to be effective in reducing mortality.

Numerous other workers including Miessner and Schern, Frohner und Zwick, Wiemann J., and others describe ovine pasteurella infections and allied conditions. A few years ago Schütze (1929) gave a comprehensive review of pasteurellosis.

HISTORY OF THE PRESENT OUTBREAK.

In December 1931 the experimental farm of the University of Pretoria imported two Ryeland rams from England, and a year later five ewes of the same breed. The imported sheep were kept with a number of Merinos on the University farm. All were pastured under identical conditions in the same camps, fed from the same food and housed in the same buildings. During the winter of 1933 one of the rams (ram No. 1) became unthrifty and developed a short dry cough. It was treated for bronchitis and kept under observation. It improved slightly and served a few ewes, but in March, 1934, it suddenly developed acute tympanites associated with intense pulmonary distress from which it died within twenty-four hours. An autopsy revealed marked lesions of necrotic fibrinous pleuro-pneumonia, hydrothorax and hydropericardium, acute catarrhal gastro-entritis, tracheitis, pharyngitis and laryngitis, enlargement of the liver and spleen, and degenerative changes in the kidneys.

Cultures were made from the heart-blood and pneumonic areas. No growth resulted in the tubes inoculated with heart-blood, but several smooth whitish-grey, moist-looking, translucent colonies of different sizes appeared on the serum-agar slants inoculated with material from the lungs. A few single large colonies were picked, subcultured and studied. The organisms of the different colonies appeared to be much alike so that the growth obtained from only one colony was studied in detail. This culture was designated *pasteurella* 182 and is described below.

A week after the death of ram No. 1 the second ram (ram No. 2) also developed symptoms of acute pneumonia and died about fifteen hours after it was first noticed sick. An autopsy again revealed lesions of fibrinous pleuro-pneumonia, hydrothorax, hydropericardium, and inflammation of the pharynx, larynx and trachea. Both lungs were involved and there were fibrinous adhesions between the pulmonary and costal pleura. There was gastro-enteritis, degeneration of the kidneys, and enlargement of the spleen and liver. Cultures were again made from the heart blood and lung lesions and as in the case of ram No. 1 several greyish-white, moist, smooth, translucent colonies of different sizes developed on the media inoculated with the material from the lungs, but the tubes seeded with heart blood remained sterile. Morphologically the organisms from the different colonies were indistinguishable, and finally a culture obtained from one of the smaller colonies was kept and studied in detail, the others being discarded. This culture was called *pasteurella* 181 and is described below.

As a result of the death of both these animals the University was left without any male breeding stock and it was hoped that a purebred ram lamb (ram No. 3) the progeny of ram 2 now about five months old would soon be available for breeding purposes. As the five Ryeland ewes and their progeny, including ram 3, did not thrive so well as the locally bred Merinos it was thought advisable to inoculate them with a vaccine made from culture 182, the vaccine used being a formolised saline emulsion of a twenty-four hours old serum-agar growth. All the sheep were inoculated once only. Soon afterwards all the ewes improved remarkably in condition, but ram 3, although showing a slight improvement, remained unthrifty. Early in August it suddenly became worse and developed acute pneumonia from which it died the following day. The most outstanding lesions found on autopsy were fibrinous pleuro-pneumonia, hydropericardium, hydrothorax, fatty degeneration of the liver and kidneys, gastro-enteritis and tumor splenis.

Cultures were made from the heart-blood, liver, spleen, lung lesions and pleuritic fluid. Numerous smooth translucent, shiny, greyish-white, moist colonies of varying sizes were observed on media inoculated with material from the lungs; the liver and pleuritic fluid also yielded growths. A number of the larger and some of the smaller colonies were picked, subcultured and studied. In morphology and staining characteristics the organisms from the large and small colonies were indistinguishable, but in virulence and bio-chemical reactions they showed noticeable differences. The organisms from the large colonies were apparently identical and a culture from only one (*pasteurella* 247) was kept for further study; great differences, however, were observed between the cultures obtained from two of the small colonies and between them and those obtained from the large colonies. The two small colonies studied yielded cultures 247a and 247c. The colonies found on the media seeded with liver were similar to the large colonies produced on the serum-agar by the lung material; the culture obtained from the pleuritic fluid became contaminated with *B. subtilis* and was discarded.

BACTERIOLOGY.

(1) PASTEURELLA 182.

Morphology and Staining Characteristics.

The organisms were generally small, ovoid and irregularly arranged in smears, sometimes pleomorphic, some being short bacillary (ovoid) in form while others were long and filamentous. They were non-motile. Bipolar staining was common and gave the bacilli a characteristic appearance, especially in very young cultures and in blood smears made from cases of septicaemia. In some cultures, however, the bipolar staining was not apparent. The organisms were Gram-negative.

Cultural Characteristics.

There was a moderate growth on most of the ordinary laboratory media. Freshly isolated cultures grew fairly well on nutrient agar, but after repeated subcultivation the growth became poorer and poorer. On serum-agar a much more abundant growth was obtained. Single colonies were raised, greyish-white, clear, translucent, moist, smooth, and spread peripherally during the course of a few days' growth. Fresh cultures could be washed off readily with saline, but older ones became dull and viscous, and tended to adhere to the medium. Saline emulsions, even when made from fresh cultures, were generally flocculent. On solid media a maximum growth was usually obtained after two to three days incubation.

In broth a uniform turbidity was formed after twenty-four hours and a white, powdery, sometimes flocculent deposit collected at the bottom of the tube. The growth continued in broth for weeks so that in the course of a fortnight, or sooner, a thick flocculent deposit was found at the bottom, leaving the supernatant fluid almost clear. The deposit disintegrated with difficulty and it required a great deal of shaking before a uniform emulsion could be obtained. Sometimes a pellicle formed on the surface of the fluid. On blood agar there was no haemolysis and gelatin was not liquified.

Biochemical Reactions.

Acid without gas was formed in glucose, saccharose, mannite and maltose. Litmus milk was not altered, the methyl-red and Voges-Proskauer reactions were negative, while the methylene blue reductase and catalase tests were positive. Nitrates were reduced, ammonia and a small amount of hydrogen sulphide as well as indol were formed.

Virulence Tests.

(a) *For Guinea-pigs.*

Guinea-pigs 1 to 14 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1 and 2.............	Intraperitoneal	1/20 of a 24-hours old serum agar slant.
3 and 4.............	,,	1/200 of a 24-hours old serum agar slant.
5 and 6.............	,,	1/2000 of a 24-hours old serum agar slant.
7 and 8.............	,,	1/20000 of a 24-hours old serum agar slant.
9, 10, 11, 12.........	,,	1/200000 of a 24-hours old serum agar slant.
13 and 14...........	,,	0·05 c.c. of a 24-hours old broth culture.

Guinea-pigs 1, 2, 3, 4, 5, 6, 7, 13, and 14 were dead within twenty-four hours. Guinea-pigs 8 and 9 died after forty-eight hours, while 10, 11, and 12 survived for a week when they were reinoculated with the same dose of organisms as before. Within forty-eight hours all were dead. All the dead guinea-pigs showed lesions of severe fibrinous peritonitis, tumor splenis and enteritis, while some showed in addition hydrothorax and hydropericardium. *Pasteurella* in pure culture were obtained in media inoculated with heart-blood from guinea-pigs 5, 7, 8, 9, 10, 11, and 13. No cultures were made from the others.

Subsequently two more guinea-pigs were inoculated intraperitoneally with 1/200,000 of a twenty-four hours old serum-agar culture. Both died within forty-eight hours showing typical lesions of pasteurellosis and both yielded pure cultures of *pasteurella* in media inoculated with heart-blood.

(*b*) *For Rabbits.*

Rabbits 1 to 5 were inoculated as follows:—

Rabbits.	Method.	Material.
1	Intravenous	1 c.c. of a 24-hours old broth culture.
2	,,	2 c.c. of a 24-hours old broth culture.
3	,,	1/10 of a 24-hours old serum agar culture.
4	,,	1/100 of a 24-hours old serum agar culture.
5	,,	1/1000 of a 24-hours old serum agar culture.

Rabbit 3 died after a week, but *pasteurellae* could not be found in media inoculated with heart-blood and no outstanding lesions were revealed at autopsy. Two more rabbits were inoculated with 1/10 of a twenty-four hours old serum-agar culture; one of these died after four days, but again heart-blood cultures remained negative for *pasteurellae* and no lesions were observed. The other rabbits all remained healthy.

(*c*) *For Pigeons.*

Pigeons 1 and 2 were inoculated respectively with 1/10 and 1/100 of a twenty-four hours old serum-agar culture. Pigeon 1 died after forty-eight hours, but pigeon 2 survived. The inoculations were made intramuscularly

(*d*) *For Sheep.*

Sheep 37285 was inoculated intrapulmonarily with 10 c.c. of a twenty-four hours old broth culture of *pasteurella* 182 (15.3.34). On March 16th and 17th it was listless with an elevated temperature (107·5° F.) and accelerated respiration. On March 18th it died and an autopsy revealed a necrotic fibrinous pleuro-pneumonia with hydrothorax and hydropericardium. Culture media seeded with heart-blood yielded a pure culture of *pasteurella*.

Sheep 28078 was injected intrapulmonarily (19.3.34) with 2 c.c. of a twenty-four hours old broth culture. After twelve hours the animal became listless, its temperature shot up to 108° F. and its breathing became rapid and distressed. The temperature suddenly dropped to 106° F. on the next day and then to 104° F. on the day after that. On the fourth day the temperature was 102° F. and on the sixth day the animal died. The most outstanding lesions presented were hyperaemia and oedema of the lungs, and hydropericardium. The carcase was fairly decomposed at the time of autopsy and no cultures were made.

Sheep 38880 was inoculated intrapulmonarily (25.3.34) with a 2·5 c.c. of a twenty-four hours old broth culture. On the eighth day the temperature rose to 106° F. remained at that level for two days and then dropped to 103° F. on the tenth day a few hours before the animal died. Symptoms of listlessness and distressed breathing were also manifested during the febrile stage. An autopsy revealed lesions of fibrinous pleuritis, slight pericarditis, icterus and gastroenteritis. *Pasteurellae* were obtained from the media seeded with heart-blood.

Sheep 37744 was inoculated intravenously (15.3.34) with 10 c.c. of a twenty-four hours old broth culture. The temperature shot up to 108° F. within twelve hours and the animal was found dead on the following day, showing lesions of pulmonary congestion and oedema, and epicardial haemorrhages. A pure culture of *pasteurella* was obtained from the heart-blood.

Sheep 32606 was inoculated intravenously with 2 c.c. of a twenty-four hours old broth culture. On the fourth day the temperature suddenly shot up to 107° F. while the animal became listless and showed rapid distressed breathing. During the following two days the temperature dropped, reaching 103·2° F. a few hours before the sheep died on the sixth day. Cultures made from the heart-blood yielded *pasteurellae* and the chief lesions observed were fibrinous pleuro-pneumonia, atelectasis of both lungs and enteritis.

Sheep 33993 was inoculated intravenously (22.3.34) with a saline emulsion of half a twenty-four hours old serum-agar culture. On the following day the temperature was 105·6° F. and the breathing distressed. After the first day the temperature rapidly dropped and was 103° F. a few hours before the animal was killed in extremis on the fourth day. The only pathological changes observed at autopsy were atelectasis of the right lung, enteritis and enlargement of the liver.

Sheep 36988 was inoculated intravenously with 1/10 of a twenty-four hours old serum-agar culture. On the following day its temperature had risen to 106·5° F. and its breathing was very rapid; on the second day the temperature had dropped to 102° F. and after that it fluctuated for several days between 102° F. and 105° F. Finally the temperature remained regular and the animal recovered.

Sheep 34333 was inoculated intravenously with 1/50 of a saline emulsion of a twenty-four hours old serum-agar culture. No obvious disturbance of health was noticed at any time.

The lungs and spleen of ram 2 were passed through a Latapie mincer and the emulsion obtained was utilised as follows:—

Sheep 27240 was inoculated intravenously (14.3.34) with 10 c.c. of the Latapie emulsion. On the second day the temperature was 105·6° F. and then fluctuated between 105·5° F. and 104° F. until the tenth day when it rose to 106·4° F. and on the twelfth it was 106·8° F. After this the temperature gradually dropped to 103° F. and the animal died on the twenty-fifth day. The most outsanding lesions recorded were purulent pleuro-pneumonia, (there were fractures of ribs on the right side with laceration of the corresponding lung) hydropericardium, fatty degeneration of the liver, and kidneys, and atelectasis of the right lung.

Sheep 38914 was injected intrapulmonarily (14.3.34) with a 10 c.c. of the Latapie emulsion. The temperature rose to 106·5° F. within twenty-four hours and then dropped to 102° F. on the second day; on the thirteenth day the temperature again rose to 106·8° F. and dropped on the following day as before. The sheep finally recovered.

On March 16th *sheep* 37698 was inoculated intravenously with 10 c.c. of a Berkefeld filtrate of the Latapie emulsion. On the second and fifth days the temperature suddenly rose to 105° F. and subsided on the following days. The animal showed no further reaction and remained healthy.

On March 16th *sheep* 37417 was inoculated into the right lung with 10 c.c. of a Berkefeld filtrate of the Latapie emulsion. Apart from a moderate rise in the temperature the sheep remained healthy.

Sheep 37831, 37818, 37798 were kept as controls in close contact with the above experimental animals. All remained healthy.

Immunising Properties.

An atempt was made to immunise laboratory animals with either formolised or live cultures of *pasteurella* 182. Guinea-pigs were inoculated with formolised saline emulsions of serum-agar cultures or with formolised broth cultures and rabbits were injected with live emulsions.

Eighteen guinea-pigs were inoculated subcutaneously at weekly intervals with progressively increasing doses of a formolised forty-eight hours old broth culture. The first dose was 0·5 c.c. and the final dose was 5 c.c. inoculated on two sides of the body. Six injections in all were given.

Six guinea-pigs were repeatedly inoculated with progressively increasing doses of a formolised saline emulsion of serum-agar cultures. The density of the emulsion corresponded to the opacity of the nephalometer tube (Burroughs and Wellcome) indicating a concentration of *B. coli* 3×10^9 per c.c. The dose varied from 0·5 c.c. to 2 c.c.

Rabbits were inoculated with increasing doses of live cultures; 1/1,000, 1/100, and 1/10 of a twenty-four hours old serum-agar slant being injected intravenously at weekly intervals, commencing with the smallest dose.

A week after the last inoculation the guinea-pigs were tested for immunity. They were inoculated intraperitoneally with various dilutions of saline emulsions made from twenty-four hours old serum-agar cultures. Those injected with amounts in excess of 1/200,000 of a culture all died, showing *pasteurellae* in the heart-blood, while about 50 per cent. of the guinea-pigs injected with 1/200,000 of a culture survived.

On account of the poor immunity produced in the guinea-pigs, it was decided to ascertain the agglutinin titre of the serum of some of the animals which had received the injections. Accordingly, four of them were bled to death and the serum separated. An agglutination test was made with the homologous organisms commencing with a dilution of 1/10. The test was entirely negative.

The same test was performed with four of the rabbits injected with the live emulsions, but apart from an incomplete agglutination in a dilution of 1 : 50 in one case the tests were negative.

The results of other workers, Schütze (1929), Cornelius (1929) also seem to indicate that the antigenic properties of pasteurellas are generally very feeble and that they are unreliable for the production of sera of a reasonable titre.

The use of precipitating sera, as described by Yusef (1935), for the recognition of pasteurellas has not been tried.

Results.

Pasteurella 182 has been found to be highly pathogenic for guinea-pigs and sheep, but rabbits and pigeons were extremely resistant to infection and withstood doses of virulent culture that proved to be lethal for sheep. The organism apparently has a predilection for pulmonary tissue and serous membranes because the lungs and pleurae were almost invariably affected extensively even when the infection was made by the intravenous route. Affected lungs passed through the Latapie mincer produced a definite reaction in sheep when the injection was made either intravenously or intrapulmonarily. The animal inoculated by the former route died from fibrinous pleuro-pneumonia, while the sheep that received the emulsion by the latter route recovered. Berkefield filtrates of the Latapie emulsions were harmless for sheep.

The immunity produced in guinea-pigs by repeated injections of formolised cultures was negligible, and in both guinea-pigs and rabbits inoculated with dead and live cultures respectively, appreciable amounts of agglutinin could not be demonstrated.

(2) PASTEURELLA 181.

In morphology, and staining characteristics this organism could not be differentiated from *pasteurella* 182. Cultures of *pasteurella* 181 on serum-agar retained the moist, translucent appearance longer than the other organism, they became less adherent to the underlying medium and did not develop the dullness so early. The primary freshly isolated colonies were smaller than the single colonies of *pasteurella* 182, but on subculturing the colonies became larger.

Biochemical Reactions.

Fermentation of sugars was found to be irregular; generally acid but no gas was formed in glucose, saccharose, maltose, mannite, and inosite. Indol was not formed, nitrates were not reduced and the methyl-red and Voges-Proskaues tests were negative; but ammonia and hydrogen sulphide were formed. The methylene blue reductase and catalase tests were both positive, but litmus milk remained unaltered.

Virulence Tests.

(*a*) *For Rabbits.*—Rabbit 1 was inoculated intravenously with 1 c.c. of a twenty-four hours old broth culture and rabbit 2 was inoculated with a 2 c.c. of the same culture.

(*b*) *For Guinea-pigs*.—Guinea-pigs 1 to 5 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1	Intraperitoneal	0·05 c.c. of a 24-hours old broth culture.
2	,,	0·1 c.c. of a 24-hours old broth culture.
3	,,	0·25 c.c. of a 24-hours old broth culture.
4	,,	0·5 c.c. of a 24-hours old broth culture.
5	,,	1 c.c. of a 24-hours old broth culture.

After forty-eight hours guinea-pigs 4 and 5 were dead, *pasteurellae* being recovered from cultures made from the heart-blood. Both the rabbits and the other three guinea-pigs remained healthy. The test was repeated on guinea-pigs with saline emulsions of serum-agar cultures, but death resulted only in those animals that received very large doses—1/5 and 1/10 of an agar slant.

Results.

Pasteurella 181 was only very slightly pathogenic for guinea-pigs, and rabbits remained unaffected even by employing comparatively large doses of virulent culture. It differed from *pasteurella* 182 culturally, biochemically and in virulence.

(3) PASTEURELLA 247.

In morphology, cultural and staining characteristics this organism was found to be identical with *pasteurella* 182.

Biochemical Reactions.

Like strain 182 this organism formed acid but no gas in glucose, saccharose, mannite and maltose. It did not change litmus milk or liquify gelatin. It gave negative methyl red and Voges-Proskauer tests, but positive methylene blue reductase and catalase reactions. It reduced nitrates, formed ammonia, a small amount of hydrogen sulphide, and indol.

Virulence.

In virulence also this organism closely resembled pasteurella 182.

Virulence Tests.

(*a*) *For Guinea-pigs.*

Guinea-pigs 1 to 6 were inoculated as follows:—

Guinea-pigs.	Method.	Material.
1	Intraperitoneal	0·5 c.c. of a 24-hours old broth culture.
2	,,	0·1 c.c. of a 24-hours old broth culture.
3	,,	1/20,000 of a 24-hours old serum agar slant.
4	,,	1/20,000 of a 24-hours old serum agar slant.
5	,,	1/200,000 of a 24-hours old serum agar slant.
6	,,	1/200,000 of a 24-hours old serum agar slant.

After forty-eight guinea-pigs 1, 2, 3, 4, 5, were dead, showing lesions of hydrothorax, hydropericardium and intense fibrinous peritonitis; *pasteurellae* were obtained in pure culture from the heart-blood of the dead animals. Guinea-pig 6 survived. Later four more guinea-pigs were inoculated each with 1/200,000 of a twenty-fuor hours old serum-agar slant; of these three died.

(*b*) *For Rabbits.*

Rabbit 1 was inoculated intravenously with 1/10, rabbit 2 with 1/100, and rabbit 3 with 1/1,000 of a twenty-four hours old serum-agar slant. After forty-eight hours rabbit 1 was dead, but no *pasteurellae* were found in media inoculated with heart-blood and no outstanding lesions were detected in the carcase. Two more rabbits were inoculated each with 1/10 of a twenty-fours hours old serum-agar slant and the result was the same as before. Rabbits 2 and 3 survived.

(*c*) *For Pigeons.*

Pigeon 1 was inoculated intramuscularly with 1/10 and pigeon 2 with 1/100 of a twenty-four hours old serum-agar slant. After forty-eight hours pigeon 1 was dead, but pigeon 2 survived.

(*d*) *For Sheep.*

Sheep 40531 was inoculated intravenously (8.10.34) with half the emulsion from a twenty-four hours old serum-agar slant of *pasteurella* 247. On the following day the temperature was very high (106·8° F.) and the animal showed distressed and rapid breathing. The symptoms persisted for five days when the animal died. An autopsy revealed intense fibrinous pleuritis, marked oedema and congestion of the lungs, hydrothorax, hydropericardium, fatty degeneration and bile pigmentation of the liver and enteritis. Cultures were made from the heart-blood, liver, pleuritic fluid and lungs, from all of which *pasteurellae* were obtained.

Sheep 40444 was inoculated intravenously (8.10.34) with 1/10 of a twenty-four hours old serum-agar slant. Within twenty-four hours the temperature rose to 107·7° F. but dropped to normal on the following day; on November 2nd and 9th the temperature again rose to 105° F. After this the temperature dropped and the animal recovered.

Sheep 40994 was injected intratracheally (17.10.34) with 1/10 of a serum-agar slant; the same dose was repeated after a week. The sheep remained apparently healthy.

Sheep 41034 was injected intratracheally (17.10.34) with one-half of a twenty-four hours old serum-agar slant, the same dose being repeated after a week. A certain amount of the first dose was expelled through the nose while the animal was being drenched. The day following the second injection the temperature rose to 106° F. and then gradually subsided for a week until it reached 102° F. After this the temperature rose periodically to 105° F. but the animal recovered.

Sheep 40488 was drenched (15.10.34) with 20 c.c. and sheep 40538 with 450 c.c. of a forty-eight hours old broth culture of *pasteurella* 247. Both animals remained apparently well.

Sheep 40414 was drenched (12.10.34) with about 100 c.c. of an emulsion made from the lungs of sheep 40531 (which died from an intravenous injection of *pasteurella* 247). On the tenth day its temperature rose to 107° F. and then suddely dropped to 102° F. on the following day; on the twenty-second day the temperature rose to 105·4° F. and again dropped to 102° F. within twenty-four hours; on the thirty-first day there was another rise to 105° F., after which the temperature gradually dropped to 101·8° F. and no further reaction occured.

Sheep 40777 was drenched on (12.10.34) with about 100 c.c. pleural fluid from sheep 40531 (which died from an intravenous injection of *pasteurella* 247). On the thirteenth day the temperature suddenly rose to 107° F. and then as suddenly dropped to 103° F. on the following day. After this the animal remained healthy.

Results.

Pasteurella 247 was shown to be pathogenic for guinea-pigs and sheep, both rabbits and pigeons being remarkably resistant to infection. In guinea-pigs infection was very readily set up by intraperitoneal inoculations, while in sheep death was produced by the intravenous route. Although a fatal infection did not result from an intratracheal injection of virulent material, sheep so injected showed a severe thermal reaction. When virulent cultures were given *per os* no reaction was observed, but when organ material from an experimentally infected sheep was given to two sheep by the same route a definite thermal reaction was set up in both cases.

The organism apparently has a predilection for pulmonary tissue and serous membranes as the most outstanding lesions developed in in the lungs and pleurae, even when virulent material was given intravenously.

(4) PASTEURELLA 247 (*a*).

In morphology and staining characteristics *pasteurella* 247 (*a*) was indistinguishable from *pasteurella* 247 but differed from it culturally, biochemically, and in virulence. In these respects it resembled *pasteurella* 181. Primary colonies of this organism on serum-agar were much smaller than those of *pasteurella* 247. After subculturing single colonies became much larger.

Biochemical Reactions.

Pasteurella 247 (*a*) formed acid but no gas in glucose, saccharose, mannite, maltose, and inosite. It did not change litmus milk, the methyl-red and Voges-Proskauer tests were negative; indol was not formed and nitrates were not reduced; ammonia and hydrogen sulphide were formed in small quantities; the methylene blue reductase and catalase reactions were positive.

Virulence Tests.

Guinea-pig 1 was inoculated with 0·1 c.c. of a twenty-four hours old broth culture, guinea-pig 2 was injected with 0·25 c.c. and guinea-pig 3 with 0·5 c.c. Guinea-pig 3 died after forty-eight hours, but Nos. 1 and 2 remained healthy. The test was repeated with three more guinea-pigs with similar results. *Pasteurellae* were recovered from the heart-blood of the dead animals.

These results indicated that *pasteurella* 247 (*a*) resembled *pasteurella* 181 not only in morphology, cultural and staining characteristics, but also in biochemical reactions and virulence. It cannot be regarded as identical with *pasteurella* 247 and 182.

(5) PASTEURELLA 247 (*c*).

In morphology and staining characteristics *pasteurella* 247 (*c*) was also very much like pasteurella 247, but differed from it culturally, biochemically and in virulence.

Although this organism also grew best on serum-agar it grew only moderately well in broth and poorly on nutrient agar; but the colonies were very much smaller than those of *pasteurella* 247 and a much weaker growth was obtained on laboratory media, including serum-agar.

Biochemical Reactions.

No change occured in litmus milk, indol was not formed, the Voges-Proskaues and methyl-red tests were negative, nitrates were very slightly reduced, and ammonia was formed in very small quantities. the methylene blue reductase test was weakly positive and the catalase test was negative. Sugars were not fermented at all. The growth on sugars and on the various media used for biochemical tests was very poor, a fact which possibly explained the absence or mildness of reactions.

Virulence Tests.

Guinea-pig 1 was injected intraperitoneally with 0·5 c.c. of a twenty-four hours old broth culture and guinea-pig 2 with 1·0 c.c. of the same culture. Both animals remained healthy.

These results show that a third and entirely different type of *pasteurella* was obtained from the cultures made from the lungs of ram 3. It differed from strains 247, 247(a), 182, and181, and it was the least pathogenic of all the strains isolated and studied.

CLINICAL OBSERVATIONS.

These are confined to the three natural cases seen in the Ryeland rams and the cases of pasteurellosis produced in sheep by the injection of strains 182 and 247 of the organism. Of the three natural cases seen one was noticed to be sick for only twenty-four hours before death occured. The other two showed symptoms of pneumonia for periods of two to three weeks before succumbing. Individual experimental sheep varied clinically to a large extent apparently depending upon the amount of material and the method used. For example, sheep 37744 received 10 c.c. of a broth culture on the afternoon of 15.3.34 and died on the next day, the disease assuming a septicaemic form. On the other hand, sheep 27240 received 10 c.c. of a lung emulsion from one of the early cases on 14.3.34 and died from pleuro-pneumonia on 9.4.35 nearly a month after the injection.

In the animals that died from the septicaemic or acute form of the disease symptoms observed consisted of severe respiratory distress with accelerated breathing and pulse rates and marked hyperthermia. Within twenty-four hours the animals were dead. A number of the experimental sheep, however, as well as two of the natural cases showed considerable similarity in clinical symptoms. The high febrile reaction occuring a few days after the injection was characteristic and it was always associated with accelerated respiration and pulse rate; the breathing in addition becoming distressed. After reaching its highest point usually between 107° and 108° F. the temperature always dropped to about 104° F. shortly before death.

Sheep 32606 which received 2 c.c. of a broth culture intravenously on 19.3.34 may be taken as well representative of the usual case seen. The temperature remained between 102° and 104° F. up till the morning of 22.3.34, when it commenced to rise and by the afternoon of the 23rd it had reached 107° F. Then it dropped almost as suddenly as it had risen and was 104° F. on the afternoon of 24.3.34 shortly before the animal's death on the 25th. On the 23rd the animal remained down with a greatly accelerated pulse and respiration rates, and had a slight bilateral muco-purulent nasal discharge. In the animals which recovered an intermittent fever was maintained for periods of up to three or four weeks. The temperature fluctuated between 103° F. and 108° F. eventually reaching a normal level. During the period of fever the pulse and respiration rates were increased but dropped again as the fever decreased.

Whilst it would be unwise to make a diagnosis of pasteurellosis upon clinical grounds alone the symptoms seen can be usefully correlated with *post-mortem* appearances and laboratory examinations.

PATHOLOGICAL ANATOMICAL CHANGES.

These are confined to the *post-mortem* appearances seen in sheep; both in the natural cases from the University farm and in the cases artificially produced with strains 247 and 182 of the organism and ending fatally. They may be roughly classed with those seen in sheep that died from the septicaemic or acute form i.e. within one or two days and those seen in animals which were sick for a week or more.

Changes in the animals which died from the acute or septicaemic form were limited to severe hyperaemia and often oedema of the lungs with enlargement of the spleen. The latter change was never very severe, the largest spleen seen measuring 10 × 7·5 × 2 cms. Hydrothorax and hydropericard were usually seen, and in some instances there was a hyperaemia of the mucous membrane of the gastro-intestinal tract. Slight fatty degeneration of the liver and kidneys was noticed in these cases. No emaciation was seen except in the case of the third Ryeland ram which was losing condition for some months before it developed acute pneumonia and died suddenly.

In the animals which died after a more lengthy course of the disease, much more significant and severe lesions were noted at autopsy. They usually showed some degree of emaciation; those that had been ill for some time were often very poor in condition. In one sheep (27240) two ribs on the right side were fractured but this was apparently the result of a traumatic injury. The changes which may be classed as occurring almost invariably were those in the respiratory tract. There was frequently a muco-purulent discharge from the nostrils and in many cases a severe hyperaemia of the pharynx, larynx and trachea. The lungs and pleural membranes were the seat of severe damage in almost every animal, either fibrinous pleuro-pneumonia or purulent broncho-pneumonia being present. Usually the lung was found partially deflated and heavier than normal. It was firm to the touch and of a mottled bluish-red or light red colour. The pleural membranes, both costal and pulmonary were frequently covered with a fibrinous yellow deposit up to 2 cms. in thickness and the lung was often attached by fibrinous material to the costal wall over varying areas. A large amount of turbid yellow or red fluid was usually present in the thoracic and, to a lesser extent, the pericardial cavity.

On sectioning the lung, which cut like a solid organ such as liver, the cut surface varied in colour from a light pink to a dark red, often with lighter coloured yellow areas. A reddish turbid fluid could be expressed and was present in the bronchioli. Frequently light coloured areas of soft necrotic tissue were seen. The whole of the lung tissue was never hepatised—the parts usually affected being the anterior and inferior parts of both lungs—and the junction between the normal and healthy tissue was always clearly demarcated. In some of the cases parts of the lungs were atelectatic, being completely collapsed and dark red in colour.

Changes in the other organs were limited to fatty degeneration of varying severity in the kidneys and liver and occasionally a slight enlargement of the spleen. The gastro-intestinal tract sometimes showed hyperaemia in the mucosa.

HISTO-PATHOLOGICAL CHANGES.

In every case that terminated fatally specimens of internal organs were collected and fixed in formalin; sections were cut by either the freezing or the paraffin embedding method. Organ smears were also made and stained with Giesma. The stains used in the sections were Haematoxylineosin, van Giesen, Sudan III, and Giemsa.

The sheep that died from the acute form of the disease showed very few histological changes: the blood content of the spleen was increased and the lymphoid follicles enlarged. Bipolar organism were demonstrated without difficulty in spleen smears from these cases.

In the lungs hyperaemia and oedema was observed. The alveoli were filled with clear serous material and the capillaries were distended with blood.

In animals that lived a week or more after the infection characteristic changes developed in the respiratory organs. In the lungs a constant feature was the severe hyperaemia present. Every capillary blood vessel was packed with blood cells. In almost every case areas of consolidation were present. The alveoli in these areas were filled with a mass of fibrinous material, neutrophiles and red blood corpuscles. The fibrin was in the form of a network of very fine strands in which the infiltrating cells and red corpuscles were enmeshed. Large phagocytic cells (macrophages) were also present. The fibrin, rather difficult to identify with the Haematoxylin-eosin stain was very clearly shown up by Wiegert's method (Fuchsin, Methylviolet and Lugol's iodine). In these cases of fibrinous pneumonia in sections cut by the paraffin method and stained with Giemsa numerous bacteria were present amongst which bipolar staining organisms could be clearly identified. Necrotic areas were seen in which all cell structure had disappeared and which showed up as light pink staining (Haematoxylin-eosin) homogeneous structureless foci surrounded by a zone of neutrophiles.

Cases were seen in which as well as a fibrinous pneumonia, areas of purulent broncho-pneumonia were also present. Here the alveoli contained no fibrin but were blocked with a mass of neutrophiles and large phagocytic cells. In some of the sheep this type of pneumonia only was present, and no bipolar organisms were seen, although numerous cocci and short bacillary organisms could be demonstrated.

In some sheep the pneumonic changes were accompanied by a fibrinous pleuritis. In sections the pleural membrane of both the lung and the costal wall was roughened and thickened and covered with a dense layer of fibrin which again appeared on a network of fibrils enclosing here and there red blood corpuscles and a few neutrophiles. In the sections stained with Giemsa bipolar organisms, as well as other bactera, could be demonstrated fairly easily. In smears made from the consolidated parts of the lungs numerous bacteria were present including frequent bipolars.

The histological changes in the lung can then be summarised as including acute and sub-acute fibrinous and purulent broncho--pneumonia, frequently with areas of necrosis and fibrinous pleuritis.

In other organs such as liver and spleen a hyperaemia was occasionally noted, whilst in the liver and kidneys fatty degeneration to a greater or lesser extent was usually present.

DISCUSSION.

A virulent form of pasteurellosis in Ryeland sheep at the experimental farm of the University of Pretoria was investigated. Of the three natural cases studied, two had been suffering from a chronic pulmonary infection for several weeks before they died while the third (ram 3) was not noticed sick for more than twenty-four hours before death. Ryeland ewes kept under identical conditions and in close contact with the rams did not suffer in the same way. It is true that at one time these ewes were unthrifty and that they improved in condition after they had been inoculated with a formolised emulsion of *pasteurella* 182, but there is no proof that they suffered from pasteurellosis or that the improvement could be attributed to the inoculation; ram 3 which received the same treatment finally succumbed to a pulmonary disease from which an organism (*pasteurella* 247) identical with *pasteurella* 182 was obtained.

Primary cultures made from the lungs of ram 1 produced a growth containing several colonies of different sizes; one of the largest of these was picked and subcultured for a detailed study. It yielded culture 182 which was composed of small ovoid (short bacillary), bipolar-staining, Gram-negative organisms corresponding to the description of *pasteurellae* given by Topley and Wilson (1929). This organism proved to be highly pathogenic for guinea-pigs and sheep, but apparently non-pathogenic for rabbits and pigeons; it was found to have a predilection for pulmonary tissue and serous membranes; when a live culture was injected intravenously into sheep pathological changes developed in the lungs, pleurae and pericardium in preference to other parts of the body. In the three natural cases studied the lungs, pleurae and pericardium were affected most extensively. Moreover, the lesions presented by the experimentally produced cases closely resembled those observed in the natural cases.

Repeated inoculations of formolised cultures of *pasteurella* 182 did not appreciably increase the resistance of guinea-pigs to infection with virulent live cultures and the serum of the "immunised" guinea-pigs was devoid of any agglutinin content. Likewise no agglutinins could be demonstrated in the serum of rabbits inoculated with live cultures.

On serum-agar media seeded with material from the diseased lungs of the second ram several colonies of different sizes developed; one of the smaller colonies was picked, subcultured and studied. The organisms of this culture (*pasteurella* 181) were also ovoid, bipolar-staining and Gram-negative; but although morphologically indistinguishable from *pasteurella* 182, it differed from it culturally, in virulence and in biochemical reactions, the most striking of which was its inability to form indol and to reduce nitrates. For guinea-pigs it was barely pathogenic, very large doses being necessary for the production disease.

On account of the similarity of the pathological changes presented by these two rams and the apparent similarity of the primary growths on serum-agar seeded with pulmonary material from rams 1 and 2 it is suggested that each of the primary cultures contained at least two different types of colonies, one of them yielding virulent organisms corresponding to *pasteurella* 182 while the other gave rise to non-virulent bacteria like *pasteurella* 181. It is further suggested that the small colonies of the primary growths contained the non-virulent organisms while the large ones yielded virulent cultures. The bacteriology of the cultures obtained by seeding pulmonary material from ram 3 on serum-agar slants supports this view.

That the diseased tissues of naturally infected animals can set up a condition in sheep which is like the naturally occurring pasteurellosis is borne out by the effect of Latapie emulsions of the affected tissues of ram 2 on experimental sheep. Moreover, the fact that a severe fibrinous pleuro-pneumonia was produced in one animal suggests that the lungs contained a pathogenic organism as well as the slightly pathogenic pasteurella 181.

When ram 3 died from a condition in which the symptoms and pathological changes were apparently identical with those recorded in ram 1 and 2 another opportunity was presented for studying the bacteriology and pathology of the disease.

Serum-agar media were seeded with material from the lungs, liver and pleural cavities. The tubes inoculated with pulmonary material yielded several moist, greyish-white, smooth, translucent colonies of different sizes scattered over the surface of the media. A number of the different colonies, small and large, were picked and subcultured. The organisms obtained from the large colonies were found to be alike and a culture obtained from only one, *pasteurella* 247, was kept; the colonies that appeared on the media inoculated with liver were of the large variety and resembled *pasteurella* 247. The tubes seeded with pleuritic fluid also yielded a growth but as it was contaminated with *B. subtilis* it was discarded.

Culture 247 was found to be identical with strain 182, not only in morphology, cultural and staining characteristics, but also in virulence and biochemical reactions. Like *pasteurella* 182 it formed indol and reduced nitrates, and was also highly pathogenic for guinea-pigs and sheep, but not for rabbits and pigeons. An intravenous inoculation of sheep with this organism produced lesions which were not only similar to those presented by natural cases (rams 1, 2, and 3) but also to those observed in sheep infected experimentally with *pasteurella* 182.

An attempt was also made to set up an infection either by intratracheal injections or by dosing of live cultures. As a result of the intratracheal injections a marked thermal reaction was set up, but the oral administration, even of large quantities (450 c.c. broth culture) of live cultures had no apparent effect on the sheep. .

Several of the small colonies scattered over the serum-agar media seeded with lung material from ram 3 were picked. The cultures (247a and 247c) obtained from two of these were finally kept and studied. The organisms of the one were found to differ from those of the other, and the bacilli of both cultures differed from those obtained from the large colonies (*pasteurella* 247).

Pasteurella 247a was noticed to resemble *pasteurella* 181 not only in morphology, cultural and staining characteristics, but also in virulence and biochemical reactions. Like 181 it did not produce indol or reduce nitrates and it fermented the same sugars; it was also found to be very slightly pathogenic for guinea-pigs, comparatively large doses being necessary to cause death in the inoculated animals.

Pasteurella 247c yielded a much poorer growth than either 247a or 181. It differed from both of these culturally, in biochemical reaction and in virulence. For guinea-pigs it was non-pathogenic, even in very large doses.

That virulent and avirulent forms of *pasteurellae* can be recovered from the same organ of an animal suffering from pasteurellosis has been proved.

SUMMARY AND CONCLUSIONS.

From the three cases of pasteurellosis in sheep studied, five different strains of *pasteurellae* were obtained, 182 from ram 1, 181 from ram 2, and 247, 247a, and 247c from ram 3.

Of these strains 182 and 247 were highly pathogenic for both sheep and guinea-pigs, and almost non-pathogenic for rabbits and pigeons. Both showed the same biochemical reactions and both produced similar pathological changes in experimental animals inoculated with live cultures. They resembled each other also in morphology, staining and cultural characteristics. *Pasteurellae* 182 and 247 can therefore be regarded either as identical or so closely related that they cannot be differentiated by the methods employed. Both these organs have a predilection for pulmonary tissue and serous membranes and both produced lesions in experimental animals that could not be differentiated from those found in natural cases studied. These two organisms are considered to have been the cause of the mortality among the Ryeland sheep at the experimental farm of the University of Pretoria. An identical disease in experimental sheep was produced by the injection of organ emulsions and cultures made from the original cases from the University farm. Berkeveld filtrates of organ emulsions from natural cases did not produce the disease.

So far no success has yet been attained with immunisation tests in laboratory animals and no properly controlled immunisation experiments have been carried out with sheep.

The pathogenesis of the disease under natural conditions is still obscure.

Pasteurellae 181 and 247a cannot be distinguished from each other by the tests employed; both are very slightly pathogenic for guinea-pigs and both have the same biochemical reactions, and they agree in morphology, cultural and staining characteristics. Both have originated from small colonies picked from primary cultures of pulmonary material.

Pasteurella 247c does not resemble either of the two groups of organisms mentioned above. It is entirely non-pathogenic for laboratory animals.

These results indicate—

(1) that the small colonies picked from the primary growths on media seeded with material from affected lungs yielded cultures which were either non-pathogenic (247c) or only very slightly pathogenic (247a, 181);

(2) that the large colonies obtained from similar growths gave rise to highly pathogenic cultures (182 and 247);

(3) that when several colonies were picked from the same primary growth, highly pathogenic, slightly pathogenic and non-pathogenic cultures may be obtained e.g. cultures 247, 247a, and 247c);

(4) that if only one colony is picked from the primary growth either a highly pathogenic culture (182) or one which is barely pathogenic (181) may result.

It is possible that the non-pathogenic and slightly pathogenic *pasteurellae* occur as saprophytes in the respiratory passages of sheep in certain areas and that they invade the lungs only when the way has been paved for them by the entrance of pathogenic *pasteurellae* of the type 182 and 247. These latter enter the tissues and set up disease under conditions which have not yet been determined.

In making a bacteriological study of a case of pasteurellosis, therefore, it is recommended that several colonies of different sizes be picked from the primary growth, and that the pathogenicity of each one be studied separately. Only in this way may the presence of pathogenic *pasteurellae* be determined.

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The Isolation of Single Bacterial Cells.

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THE writer has three reasons for introducing what, to many workers, is a somewhat threadbare subject: (1) In the minds of not a few people with whom the author has discussed the matter, the impression still persists that the technique of the isolation of single bacterial cells is extremely difficult, involving months, if not years, of practice for its successful acquisition. Heller (1921) stated that she found the Barber (1914) method " wasteful of time, material, eyesight and nervous energy " in the isolation of anaerobic bacteria. There is some justification for saying that a wastage of time and energy occurs when, after the successful isolation of ten to twelve bacteria or spores, not one germinates. This point, the germination of the single germ, especially when it concerns the anaerobes has not received the same amount of attention that the actual isolation has. The latter is a simple mechanical process, the former one over which the operator has not the same degree of control. (2) A method of isolation, to be of real practical value and to enjoy a constant and wide application, must be simple and devoid of tedium and time wastage. Preferably should it be such that the worker would prefer to employ it rather than use plate or shake methods of isolation. Again, the learning of the technique should not require months of practice nor should it be of such a nature that only those people with " hands " can easily acquire it. Reyniers (1933) described a method by which beautiful micro-pipettes may be made mechanically. The writer gained the impression, wrong, he hopes, that the making of the apparatus would be one requiring considerable skill and time. Doubtless, however, to see the designer himself at work would dispel this idea. The method, used by the writer for over four years, has proved to be so simple that three colleagues, after half-an-hour's practice in pipette-making have settled down and isolated (with successful germination) single germs of *Cl. welchii* and *B. anthracis.* (3) The third reason advanced for the presentation of this note is in the nature of a plea for the more extended use of the single cell isolation method as a routine measure. The saving of labour and of time involved in obtaining definitely pure cultures and the feeling of assurance in having these pure cultures greatly outweighs the only disadvantage (in the writer's opinion) of the expenditure of the money for the micromanipulator. But even a machine costs no more than a good microscope and where the outlay is impossible a locally-made apparatus or the modification used by Malone (1918) may be employed. Finally, there is the possibility that less would be heard of bacterial mutation if cultures were first purified by the single-cell method.

The Making of Micro-pipettes.—On the making of satisfactory pipettes depends the success of isolation. The writer was quite unable to make an efficient article by the "tiny flame method" as described by Barber (1914) and Chambers (1922)). That this method gives highly satisfactory results there is no doubt; never having had the procedure demonstrated to him and the use at these laboratories of "paraffin gas" (Mansfield system) are doubtless the reasons for the failure. The electrical heater described (Mason 1933) solved the difficulty. The two elements, arranged in the form of a V, may be made broad or narrow. If narrow, the type of pipette most desirable for isolation work is easily prepared. It is advantageous that the micro-portion should not be more than 1·5 to 2 cm. long and that it should leave the thick portion of the hand drawn capillary sharply. A long tapering micro-pipette trembles easily and when raised against the cover slip in the process of isolation "gives" considerably, necessitating much adjustment of the control screws of the machine. Such a tapering pipette is liable to be pulled when the breadth of the elements is 3·0 cm. or more. Elements of 2·0 cm. give satisfactory results.

DETAILS.

1. Choose soft glass tubing with an external diameter of about 7·5 mm. and an internal diameter of about 6 mm. Cut portions about 30 cm. long. Soak for an hour or two in 5 per cent. hydrochloric acid, rinse thoroughly in tap water and then in distilled water. Allow to drain and dry. Plug both ends with wool and sterilize in the hot-air oven. Over a Bunsen flame, prepare two ordinary Pasteur pipettes from each portion of glass tubing. Store in a dust-free receptable until required.

2. Make a match-head flame by connecting, by means of rubber tubing, a fairly large bore hypodermic needle to a Bunsen burner. Carefully pull a much smaller capillary from the capillary end of the Pasteur pipette. Care should be taken to pull steadily and *out* of the flame whenever the heated portion of glass is nearly molten. The length of fine capillary pulled will vary in length from 3 to 6 cm.

3. Lower the fine capillary portion into the glowing heater, and so arrange its position that the micro-capillary comes off sharply. Whenever the glass "gives", pull gently and evenly, raising the pipette slightly in the heater. Very little practice is necessary to learn the degree of traction necessary for the making of a satisfactory article. As a rule separation of the micro-capillary portion from the distal end of the fine capillary occurs, but if not, the distal end is severed with sharp scissors.

4. The end of the micro-pipette must be turned up for a distance of about 2 mm. This is accomplished by holding it above the match-head flame. As the capillary turns up quickly, care should be exercised to ensure that too much is not bent or that too acute an angle does not occur. As the distal end of the turned-up portion is almost certain to be fused, enough is cut off with sterile scissors to leave approximately 2 mm. To ensure the patency of the opening, air is pumped, by means of hand bellows, through the pipette into alcohol. One can judge of the diameter of the hole by the size of

the bubbles produced. The type of bellows used is important. One of the writer's colleagues nearly discarded what were excellent pipettes because he was unable to blow air through. He was using the common type of barbers' bellows, made of thin rubber with intermediary bulb surrounded by string netting. Sufficient pressure was not obtainable. The use of thick walled bellows with only a small intermediary bulb (also thick walled and without the netting) showed that the opening were patent.

The completed pipette is clamped to the manipulator.

The Manipulator.—The writer uses the machine manufactured by the Zeiss company and has found it eminently satisfactory in every way. Whatever apparatus is used, no matter how simple of construction, a *sine qua non* is that the movements must be precise and steady. A jerkiness will produce poor results and loss of patience.

The moist chamber, cover slips and hollow-ground slides.—The moist chamber was prepared from an ordinary microscopic slide by luting to it (by means of sealing wax) strips of slides so as to form a chamber enclosed on three sides, its depth being 6 mm. This depth may without disadvantage be increased to 1 cm. One end is left open for the introduction and withdrawal of the pipette.

The cover slips and hollow-ground slides present no special features. The concavity of the hollowed slide is 25 mm. in diameter and the cover slips are of such a size that they cover the area with plenty of room to spare. Just prior to use, both are cleaned with a clean cloth and sterilized by flaming.

The Medium.—This will depend on the germ to be isolated but it must be capable of being transformed into a solid or semi-solid transparent condition.

The writer has used as routine horse-muscle infusion peptone agar plus 10 per cent. of a mixture of equal parts of sheep haemolysed cells and serum and a saline extract of guinea-pig liver. The preparation of the haemolysed cell and serum mixture has already been described (Mason 1934). The guinea-pig liver extract was added because it was found to have, as Tarozzi (1905) originally discovered, a stimulating effect upon the growth of anaerobes, the group of germs in which the writer was chiefly interested. Portions of the liver of a healthy guinea-pig were removed with sterile precautions and placed in thick walled test tubes. By means of a glass rod inserted through the cotton wool plug of the tube, the tissue was ground up. Enough ether was added to cover the liver and the tubes placed in an incubator at 37° C. for three to five hours. Then sufficient sterile saline solution (0·85 per cent.) was added to make, approximately, a 10 per cent. suspension of tissue. After stirring, the suspension was incubated for twenty-four hours. For use, equal parts of the serum, haemolysed cells and the clear supernatant of the liver extract were mixed, and added to the melted agar. The sterility of the mixture was ensured prior to its addition.

Care should be taken to have a very clear nutrient agar, as the presence of granules makes isolation very difficult.

The writer does not insist that the above described medium is the best that could be desired; it is mentioned because it has given excellent results with many different germs, and in particular with anaerobes. However, experience has shown that the admixture of some additional nutrient, such as raw serum, is advantageous, if not essential, in the obtaining of positive growth results even of such easily-grown microbes as *Bact. coli* and *B. anthracis*.

The Culture.—For the isolation of aerobes, young (twelve to twenty-four hours) surface cultures have been used, and suspended in broth just prior to use. The anaerobes will be discussed under the appropiate heading.

The Isolation.—Most workers isolate the single germs in droplets of broth on a cover slip, mark these drops, remove the contained germ with a new sterile pipette and break off the pipette end in a tube of broth. If ten such isolations are made, this involves the use of the same number of pipettes and the changes involved in fixing and adjusting them. What is, to the writer, a much simpler process, viz. the expelling, with one pipette, single germs on to the surface of a solid medium does not seem to have found the application it deserves. The methods described by Hewlett (1918), Hort (1920), Fortner (1930), and Koblmüller and Vierthaler (1933) allow of single cells being isolated on the surface of agar. In the case of the first three, one must inoculate a thin film of medium with the germs and then search for and mark a microbe lying free. This involves considerable time and eye strain and its consistent success would depend upon always inoculating with a bacterial suspension of a suitable density. With the last-named authors' technique, a number of single germs are mechanically removed from the edge of a young growing colony on an agar plate and the colonies resulting from them are observed and subcultured. With this method, the writer has had no practical experience.

Details of Isolation (use of a Zeiss manipulator, microscope and lenses).—The microscope is clamped to the manipulator and the prepared micro-pipette with attached hand bellows fastened in its holder. The bottom of the moist chamber is moistened with distilled water (a few drops should be placed at one end, the chamber tilted to allow excess to run off and the bottom rubbed with the clean finger to produce a thin even layer of water). A thin even layer of vaseline is deposited on the tops of the walls of the chamber. It is then fixed on the mechanical stage. The tip of the micro-pipette is centred, first under the low power lens (No. 8) and then under the higher power lens (No. 40). A No. 5 ocular is used. The open end of the turned-up tip should be the only part of the pipette in focus. The micro-portion of the pipette should now be lying approximately in the middle of the chamber. A cover slip and a hollowed slide, held in clamp forceps are sterilized in the flame and left " face " downwards in their holders. Meanwhile nutrient agar has been boiled and cooled to about 50° C. With a pipette, about 1·5 c.c. is deposited in a sterile tube and immediately enough of the serum-cells-liver mixture to make a 10 per cent. concentration is added and thoroughly mixed. With another pipette which has been opened by filing with a sharp file so as to produce a clean cut, the serum-agar mixture is sucked up and a thin micro-plate prepared. The cover slip in the forceps is tilted to an angle of about 45°-50° and a drop of the melted agar allowed to run down it. A little practice is necessary to learn how much agar has to be deposited so that it does not run right down the slip and over the end. The agar solidifies almost as it runs, and a thin transparent micro-plate is thus formed. With a very small platinum loop, a droplet of a previously prepared suspension of the bacteria to be isolated is deposited on a portion of the cover slip. It is best to place this drop on the cover slip, some distance from the agar. The slip is then placed, agar side downwards, on the moist chamber, pressing the sides lightly so that the vaseline seal is firm. The droplet of culture is focussed first with the low and then with the high power. The pipette point, having been previously centred will be just below the drop. Carefully raise the point into the culture, when the bacteria will be seen to flow in. When some dozens or hundreds have entered, lower the point and, with the mechanical stage, bring one end of the agar strip into view. Raise the point until it touches the agar; the moment of contact is easily seen. Lightly press the bellows and note the exit of bacteria. If, for example, only three emerge, it is worth while to pick up two leaving only a single germ. This is accomplished by bringing the point just under the germ, and raising it until it touches the agar. Often, the bacterium floats into the hole. If not, or if say ten germs emerge it is best not to waste time but rather to move to a new portion of agar (by means of the mechanical stage) and again try to expel only one germ. This may sound rather difficult, but actually by varying the pressure on the bellows and by allowing the pipette point just to touch the agar or to be slightly buried in it, ten to twelve isolations may be made in five to ten minutes. When one is certain that only one germ is lying free the area is ringed with a nose piece diamond marker. The writer has been accustomed to make from eight to ten isolations on one plate, arranged in two rows. Having finished the isolations, the pipette is lowered and withdrawn from the chamber, and the cover slip is carefully detached and placed over the concavity of the hollow slide. Vaseline is used to lute it in position. The slide is then incubated at 37° C. If one wishes to observe the progress of multiplication, the slide may be examined at frequent intervals, the individual germs being easily found in the ringed areas. Otherwise the slide is removed and examined after twenty-four hours and those areas containing colonies noted. The colonies may be removed in two ways, (1) by the use of the naked eye and a fine platinum loop or needle or (2) the cover slip is again placed on the moist chamber and the desired colony emulsified in a drop of broth expelled on to it from a coarse micro-pipette and the emulsified bacteria allowed to run back into the capillary and finally expelled into a tube of nutrient. With the first method one must work quickly as the agar film dries out in a matter of five minutes; with the second procedure, a fresh pipette is needed for each colony. If desired, one may suck up a portion of the emulsified colony in a micro-pipette and then, on a fresh micro-plate, carry out another series of isolations.

SOME PRACTICAL CONSIDERATIONS.

1. *The Size of the Hole in the Micro-pipette.*—Contrary to what would be expected, a hole just large enough to allow of the passage

of a bacterium has not proved suitable for isolation purposes. The germs do not flow into the pipette easily or at all and further, great difficulty is experienced in expelling them. An aperture two to five times the size of the microbe has given the best results.

2. *The Density of the Inoculum.*—This should be such that when the pipette point is raised into the inoculation droplet, each individual germ should be observed to enter. When they enter *en masse*, the suspension is too dense.

3. *The Thickness of the Agar Film and the Spacing of the Isolations.*—The film should be as thin and even as possible and its area should be such that when the cover slip is luted to the hollowed slide, no part of the agar touches the slide. The distance between each isolation should be so arranged that the resulting colony can be picked without danger of touching another colony.

4. *The Bellows.*—As previously stated, this should be made of thick rubber with no or only a small intermediate bulb. If the type with the net-covered intermediary bulb is used, the pressure can be released only by disconnecting the bellows from the pipette. One must be able to apply or release the pressure at will. Further, considerable pressure is often required to expel the germs.

5. *The Magnification Required.*—The writer has found it of advantage to use as low power an eye piece as possible, otherwise loss of definition with resultant eye strain occurs. For large germs such as the anthrax bacillus, and also for bacteria as *Cl. welchii* and *Cl. septicum* a Zeiss No. 5 eye-piece with a No. 40 objective have proved suitable (according to the Zeiss company this gives a magnification of X 200). When higher magnifications are required the oil immersion lens should be brought into use. With a suitably prepared agar film, sufficient working space is still available for this lens. A higher power eye piece (the Zeiss No. 7 or 10) should be used only to make certain that a single germ is lying in the field; it is not advised to carry out the actual isolation under such a lens. The condensor must be adjusted so that the bacteria stand out sharply. With ordinary daylight as illuminant, the writer has found that the lowering of it half to three quarters of its full distance gives the sharpest definition.

6. *Actively Motile Bacteria.*—The method detailed is not suitable for the isolation of germs such as *B. proteus;* a confluent film will be produced, rendering the work valueless. One may still employ the agar film method, but one bacterium only may be implanted on the whole surface. Rather should one isolate the microbes in droplets of liquid medium, in which case the cover slip should be carefully prepared as described in detail by Gee and Hunt (1928). Or one may employ the standard technique of isolating the germs in droplets and transferring these to tubes of medium.

7. *Percentage of Positive Results.*—With germs such as *B. anthracis*, *Bact. coli*, *B. typhosus*, *B. pullorum* and *staphylococci* one may expect six to ten of ten isolated cells to multiply. As one approaches the more " fastidious " microbes, as streptococci, the percentage of positive results drops, but the writer has had no difficulty in obtaining three to six positive results out of ten isolations of a delicately growing streptococcus.

8. *Application to Anaerobes.*—It was with this aspect that the writer was chiefly interested.

The culture to be used for isolation was, in the case of *Cl. welchii* and *tertium,* an eighteen hours' surface culture of the germ. The same could also be used for *Cl. septicum, bifermentans, sporogenes and oedematiens* but experience showed that a young (twelve to eighteen hours) serum-cells-liver broth culture gave better results. Ordinary infusion broth was boiled for fifteen minutes, then cooled, the mixture added, and after heavy inoculation (to ensure rapid growth) was incubated in a gas-filled jar. A young meat broth culture could not be used owing to the presence of tiny meat particles.

Fortner's (1930) *B. prodigiosus* technique proved to be the best method of securing anaerobiosis. A slight modification was introduced. Instead of seeding a portion of the micro-plate with culture, the whole of two to four 18 hours' agar slope cultures was smeared on the concavity of the hollowed slide. By such technique, germination of *Cl. welchii* was repeatedly observed after three to six hours' incubation. Experience showed that if multiplication had not commenced after twenty-four to thirty hours, it did not take place at all (micro-plates observed for seven days). By this modified Fortner technique, positive results were easily obtained with *Cl. welchii* (Types A, B, C and D), *tertium, bifermentans and sporogenes,* and with somewhat more difficulty with *Cl. septicum, oedematiens, tetani* and *sordelii.* However, the greatest difficulty was experienced with *Cl. chauvœi* and out of about 150 isolations, multiplication was obtained in one instance only. A considerable number of variations was introduced in attempts to induce the single germs to multiply, but without success. Among them may be mentioned the following:—

(*a*) *B. prodigiosus* was replaced by *Bact. coli* and by *B. Proteus* OX 19 and by Dr. Fortner's own strain of *B. prodigiosus.*

(*b*) On the supposition that air leakage could occur through the vaseline seal, the set-up micro-culture (with *B. prodigiosus*) was incubated in a MacIntosh's and Fildes' jar.

(*c*) Cystein hydrochloride was incorporated in the medium.

(*d*) Cystein hydrochloride was incorporated in the medium and in addition, the micro-plate was dropped on to the surface of the same medium in a Petri plate and incubation carried out in a MacIntosh's and Fildes' jar (see Frei and Hall 1931).

(*e*) The micro-plate with the isolated germs was set up as one side of the apparatus (made on a small scale) described by the writer (1930) for obtaining shake cultures of anaerobes.

(*f*) Little chambers through which hydrogen could be passed or in which pyrogallic acid and NaOH could be placed were constructed and the micro-plate luted thereon.

(*g*) The method of isolating in droplets of fluid and removing each germ to a tube of suitable medium (Robertson's meat broth plus haemolysed cells, serum and liver extract) was carried out in about 50 instances with one positive result. The tubes were incubated for at least three weeks before being discarded.

(*h*) Obtaining sporulating cultures on Dorset's egg medium as recommended by Henderson (1932) and isolating only those germs with spores did not lead to positive results.

(*i*) The use of different media did not lead to positive results. Amongst those tried were—Viljoen's and Scheuber's medium (Viljoen and Scheuber, 1927) (a liver extract plus peptone), Colebrook's ox liver tryptic digest medium and horse-flesh infusion agar plus 0·5 per cent glucose and the serum-cells-liver mixture. No positive result was got with 0·25 per cent. sloppy agar as an inoculum.

(*j*) A small circular hole was cut in a glass slide and over this were placed sterile pieces of thin celluloid, on the under surface of which single germs were isolated. These pieces were then dropped into meat broth. Not one of the isolated germs grew.

(*k*) On one occasion, eight isolations were made on an agar film whilst hydrogen was slowly bubbling into the chamber. The results were negative.

In the foregoing attempts three strains of *Cl. chauvœi* were used.

As the solid and liquid media used for isolation gave excellent results when large quantities of culture were seeded, one may presume that only a very odd germ was viable or the exposure to air during the isolation killed them.

Percentage of Positive results with Anaerobes.—With *Cl. welchii* and *tertium* from five to eight of ten implanted cells should grow; with *Cl. septicum, bifermentans* and *sporogenes* two to five; with *Cl. oedematiens, tetani* and *sordellii* two to three and with *Cl. chauvœi* about 1 per cent.

COMMENT.

It is possible that, to one unfamiliar with micro-manipulation, the technique as described may appear more difficult than it really is. In actual practice, the writer has usually been able to carry out the whole process, from the making of the pipette to the placing of the micro-culture (ten to twelve isolations) in the incubator, within 45 to 60 minutes. With large germs and with a suitable pipette, ten isolations should not occupy more than 5 to 15 minutes' time. Small bacteria entail more time in their isolation, caused chiefly by the period spent in assuring ones' self that only one microbe has been expelled. As explained, this can best be ascertained by carrying out he isolation under an oil immersion lens with a low power eye piece.

The chief difficulty that may be encountered is in inducing the single bacteria to grow. When fastidious germs are being worked with, endeavours should be made to devise media and/or conditions most suitable for their growth requirements.

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The Production of Immunity to *Cl. Chauvoei.*

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INTRODUCTION.

THE purpose of this communication is to place on record a number of experiments, having as end, the production of a vaccine which, when parenterally introduced into sheep, would consistently protect them against naturally-occuring black quarter. We have no intention of reviewing the very extensive literature on the subject; only those investigations having a direct bearing on this piece of research will be quoted.

About ten years' experience in the production of black quarter vaccine has taught one of us (J.R.S.) that a vaccine which so immunizes six experimental sheep that they all withstand one or two lethal doses of *Cl. chauvoei* culture (introduced intramuscularly), will prove satisfactory in the field. On the other hand, when two or three of six experimentally immunized sheep fail to resist the injection of one fatal dose of culture, reports on the lack of protection to the natural disease may be expected.

The work, about to be detailed, was initiated by failure of a number of vaccines to produce satisfactory immunity. The vaccine, issued from this laboratory, was made in a manner similar to that described by Viljoen and Scheuber (1927)*. For a number of years, such a prophylactic gave consistently good results both in experiments at Onderstepoort and in the field. From 1928 onwards the protection afforded by the vaccines (prepared in multiples of 40 litre quantities once or twice per month) slowly began to decrease, to such an extent that some batches had to be discarded in 1931-1932. Although every point in the preparation of the medium, in the culture for inoculation and in the treatment of vaccine was checked up, only one departure from the prescribed procedure was discovered. This was that the medium was autoclaved at 115° C. in 20-litre flasks on two successive days for four hours instead of previously for one hour on three successive days. The raising of the pathogenicity of the strain by repeated passage through guinea-pigs did not lead to an improvement in immunizing value of the prophylactic. One of us (J.H.M.) who has had some experience in the production of formol-toxoids and anacultures of *Cl. welchii*, Types A and B* (Wilsdon 1931) and of *Cl. septicum* suggested that the medium used (a liver infusion, peptone, salt and glucose plus liver particles) was unsuitable but against this there was the evidence of the good results in the past. However, in view of the good results obtained with Robertson's† (1916) meat broth in the production of toxins of other anaerobes and of the poor results obtained with liver medium with these other anaerobes (unpublished experiments of J.H.M.) it was decided to test out meat broth with *Cl. chauvœi*.

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* See appendix for description.

EXPERIMENTAL.

Strains of Cl. chauvœi *used*:—

(1) 64—Isolated from a natural case of black quarter in a heifer in Waterberg (Transvaal) in 1929.

(2) D—Originally isolated from the muscle of a bovine dead of black quarter, in Europe.

Media:—Meat broth was prepared as noted in the appendix.

Sloppy agar was a boiled extract of horse or donkey muscle, plus 1·0 per cent. peptone, 0·5 per cent. NaCl and 0·15 per cent. agar.

The routine liver medium was prepared as noted in appendix, and will be referred to as " routine medium ".

The sheep haemolysed red cells serum mixture was prepared in the manner described by Mason (1934).

Small amounts (10 c.c.-2,000 c.c.) of meat broth were autoclaved for half an hour at 115° C., i.e. the temperature was held at 115° C. for half an hour after having reached this point. Twenty-litre quantities of meat broth were autoclaved for 4 hours on each of two successive days. Small quantities were boiled for from 10 minutes to 2 hours and rapidly cooled just prior to inoculation. Large quantities (20 litres) of medium were allowed to cool for 24 hours before being seeded.

The pH of all media was adjusted to 8·2 just prior to autoclaving and usually, but not on all occasions, with all media except the routine, the pH was again adjusted to 7·6-7·8 with N/1 NaOH before boiling, or in the case of 20-litre amounts, before seeding. The reason for this was because the pH of media containing meat particles usually falls on storage.

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* Type A = classical *Cl. welchii*.
Type B = the " lamb dysentery bacillus " (Dalling, 1928).

† See appendix for preparation.

The inoculum for meat broth media was, unless otherwise stated, an 18 hours meat broth (plus haemolysed cells and serum) culture and for the routine medium, an 18 hours' von Hibler culture. The amount of the inoculation varied—for one- to two-litre quantities, 10-20 c.c. was added and for 20-litre amounts, about 50-100 c.c. was seeded over.

The length of incubation varied and will be noted under each experiment; the treatment of the culture after growth will also be noted under each experiment.

The virulent material used to test the immunity in sheep was, for meat broth antigens, a young (18 hours') meat broth (plus haemolysed cells) culture. Usually, such a culture was produced by seeding a tube of medium with the heart blood and/or liver of a guinea-pig killed by the intramuscular injection of the germ. In any case, the organisms in the test culture were never far removed from an animal passage. For testing animals vaccinated with routine vaccine, a guinea-pig passage culture in von Hibler's medium was usually employed.

Experiment 1.

To compare the immunizing power of antigens prepared (1) in meat broth plus 5 per cent. of a mixture of equal parts of sheep serum and haemolysed red cells; (2) in 0·15 per cent. sloppy agar plus the same mixture; (3) in meat broth plus the same mixture but using a different chauvœi strain; and (4) by using the 1·5 per cent alum precipitate of the anaculture prepared from (1).

Two litre quantities of media (1) and (2) were adjusted to pH 7·8, boiled for one hour, cooled and, after the addition of haemolysed cells and serum, inoculated with a guinea-pig passage culture of strain 64; to another flask of medium (1) strain D was added. After three days' incubation at 37° C., the meat was removed from those flasks containing it, and to all cultures enough formalin (40 per cent. formaldehyde) was added to make a 0·4 per cent. concentration. (In future this will be referred to as " adding 0·4 per cent. formalin "). The flasks were then incubated at 37° C. for three days. The sloppy agar culture was contaminated with a coccus, the meat broth cultures were not contaminated and all anacultures were sterile.* To 200 c.c. of the anaculture (1) 3 gm. of potash alum was added, the precipitate washed three times in saline and finally suspended in 200 c.c. of 0·5 per cent phenol-saline. Sheep received subcutaneously 10·0 c.c. of the one or the other antigen on 12.4.33 and were tested for immunity on 11.5.33. Table I records the results.

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* By " sterile " is meant that 5·0 c.c. of anaculture, in 50 c.c. of meat broth plus haemolysed sells and serum, produced no growth after 7 days' incubation at 37° C. and that 2·0 c.c. introduced, intramuscularly, into guinea pigs caused no more than a slight swelling of the leg.

TABLE I (Expt. S. 5003).

Immunizing power of Cl. chauvœi *vaccines, noted in Experiment* 1. 10 *c.c. vaccine injected s.c. on* 12.4.33. *Test with culture i.m.* 11.5.33.

Vaccine.	Sheep. Test with living culture i.m. (c.c.).			
	1·0	2·0	3·0	4·0
1. H.F. meat broth + Hm. cells (strain 64)	1 (35789) L	2 (21756) L	3 (35523) L	4 (35859) L
2. Sloppy agar + Hm. cells (strain 64)....	5 (35857) † 3	6 (35789) † 2	7 (35854) † 1	8 (37021) † 1
3. H.F. meat broth + Hm. cells (strain D)	9 (34478) † 2	10 (35521) L	11 (33611) L	12 (35850) L
4. Alum precipitate of 1	13 (31588) L	14 (37019) L	15 (35794) L	16 (35355) L

Controls : 17 (35517) 0·5 c.c............ † 1
18 (35537) 1·0 c.c............ † 1
19 (35542) 2·0 c.c............ † 1

(L = lived ; † 1, † 3 = died 1, 3 days ; H.F. = horse flesh ;
Hm. cells = sheep serum and haemolysed cells mixture.)

Result.—At the time when this experiment was carried out, the approximate lethal dose of a culture, prepared in meat broth from a guinea-pig passage culture was not known; later tests showed that 0·1 c.c. of such a culture killed a sheep in from twenty-four to forty-eight hours. On the assumption that the M.L.D. of the culture, used in Experiment 1, was of this order, it will be seen that three of the four vaccines so immunized sheep that they withstood about 40 lethal doses. A probable explanation for the failure of the sloppy agar anaculture to immunize is that the culture giving it origin was contaminated with a coccus.

Experiment 2.

To compare the immunizing power of (1) anacultures made in horse-flesh meat broth and ox-flesh meat broth, (2) the alum precipitates of these vaccines, (3) routine vaccine, and (4) the alum precipitate of a routine vaccine.

The horse and ox flesh broths, in 500 c.c. quantities were treated as noted under Experiment 1. The routine medium, in 20-litre amounts, was inoculated with a von Hibler culture of a guinea-pig passage strain and treated as noted in the appendix. Strain 64 was used throughout. Sheep received 5·0 c.c. of each vaccine subcutaneously on 29.5.33 and were tested on 21.6.33 by the intramuscular injection of culture (18 hours' meat broth culture of guinea-pig passage strain of 64). Table II records the results.

TABLE II (Expt. S. 5050).

Immunizing power of Cl. chauvœi *vaccines, noted in Experiment 2. Sheep injected s.c. on 29.5.33. Tested with culture i.m. 21.6.33.*

Vaccine.	Sheep. Tested with living culture i.m. (c.c.).			
	2·0	4·0	8·0	16·0
1. H.F. meat broth + Hm. cells..........	1 (37549) L	2 (37617) L	3 (35514) † 2	4 (26647) L
2. Alum precipitate of 1	5 (37668) L	6 (36796) L	7 (35778) † 2	8 (32944) † 2
3. Ox flesh meat broth + Hm. cells.......	9 (37456) L	10 (37651) L	11 (35536) † 2	12 (34510) L
4. Alum precipitate of 3	13 (37533) L	14 (37394) L	15 (35550) L	16 (32502) † 2
5. Routine medium. Anaculture. Flask 552.	17 (37577) † 3	18 (35774) † 2	19 (35551) † 3	20 (33116) † 1
6. Alum precipitate of 5	21 (37638) L	22 (35530) † 1	23 (35512) L	24 (34367) † 4
7. Routine medium, Seitz filtrate. Flask 552..	25 (37556) † 1	26 (35515) † 1	27 (37539) † 1	28 (35024) † 1

Controls : 29 (37524) 0·3 c.c............ † 1
30 (37200) 0·5 c.c............ † 1
31 (35799) 1·0 c.c............ † 1

(† 1, 2 = died after one, two days ; L = lived ;
Hm. cells = haemolysed sheep cells pus serum.)

Result.—On the assumption that the M.L.D. of the test culture was about 0·1 c.c., it will be seen, from the results given in Table II, that the anacultures prepared in both horse and ox flesh meat broth so immunized some sheep that they withstood 160 lethal doses of cultures. The results do not indicate whether or not the alum precipitates of these anacultures were better or worse immunizing agents than the anacultures themselves. The routine vaccine both as an anaculture and as a filtrate was definitely inferior to the meat broth vaccines. The alum precipitate obtained from the routine anaculture proved to have considerable antigenic power.

Experiment 3.

To test the immunizing power of anacultures made in (1) horse-flesh meat broth plus (*a*) 5 per cent. sheep haemolysed red cells, (*b*) 5 per cent. ox haemolysed red cells, (*c*) 5 per cent. horse haemolysed red cells, and in (2) ox-flesh meat plus (*a*), (*b*) and (*c*). This experiment was carried out because of the difficulty that would be experienced in obtaining sufficient sterile serum for 100-200 litre quantities of medium. The haemolysed cell solution was prepared by adding 80 c.c. of blood to 120 c.c. of distilled water containing 2·0 c.c. of a 10 per cent. pot. oxalate solution; excess ether was then added, the mixture shaken and finally incubated for 24 hours at 37° C. The sterility of the cell solutions was proved before their addition to the media. The media were contained in 500 c.c. flasks and a guinea-pig passage culture of *C. chauvœi*, strain 64, was used.

The treatment of the cultures and anacultures was as detailed under Experiment 1. Sheep received 5·0 c.c., subcutaneously, of the various vaccines on 7.7.33 and were tested for immunity on 25.7.33 (intramuscular injection of strain 64).

TABLE III (Expt. S. 5083).

The immunizing power of Cl. chauvoei anacultures made in horse and ox flesh meat broth plus haemolysed red cells of the sheep, ox and horse (no serum). Sheep received 5·0 c.c. of vaccine s.c. on 7.7.33; test 25.7.33.

Vaccine.	Sheep. Test with culture i.m. (c.c.).		
	1·0	2·0	4·0
1. H.F.M.B. plus			
(*a*) Sheep Hm. cells............	1 (37155) L	2 (36533) † 1	3 (37303) † 1
(*b*) Ox Hm. cells...............	4 (37756) L	5 (37229) L	6 (37384) L
(*c*) Horse Hm. cells............	7 (37702) L	8 (35793) L	9 (36541) L
2. Ox F.M.B. plus			
(*a*) Sheep Hm. cells............	10 (37290) L	11 (37684) L	12 (37894) L
(*b*) Ox Hm. cells...............	13 (37309) L	14 (37405) L	15 (37431) L
(*c*) Horse Hm. cells............	16 (37224) † 2	17 (37335) L	18 (37430) † 2

Controls : 19 (37494) 0·2 c.c............ † 1
20 (37678) 0·3 c.c............ † 1
21 (37680) 0·4 c.c............ † 1

(H.F. = horse flesh ; Ox. F. = ox flesh ; M.B. = meat broth ; Hm. cells = haemolysed red cells (no serum) ; L = lived ; † 1 = died 1 day.)

Result.—It is doubtful if the results given in Table III are really significant. It will be noticed that the vaccine made from horse-flesh plus sheep cells was poor and that plus horse cells was good whereas the reverse held good with the use of ox flesh. A repeat test with the same first-mentioned two vaccines gave results comparable with those reported in Table III.

Experiment 4.

To test the immunizing power of anacultures made in horse-flesh meat broth plus (*a*) 5 per cent. sheep serum (*b*) 5 per cent. of a mixture of equal parts of sheep serum and sheep haemolysed red cells, and (*c*) 5 per cent sheep haemolysed red cells. The serum was obtained from the clot and the haemolysed cells as noted under Experiment 3. Ether was added to these three products and incubated with them for 24 hours. Sterility was proved prior to their addition to the media. Flasks of 500 c.c. capacity were used, these and the anacultures being treated as noted under Experiment I. Sheep received 5·0 c.c. of vaccine subcutaneously on 16.8.33 and were tested for immunity on 30.8.33 by the intramuscular injection of a guinea-pig passage strain of 64. Table IV records the results.

TABLE IV (Expt. S. 5122).

The immunizing power of Cl. chauvœi *anacultures made in horse-flesh meat broth plus sheep serum, sheep haemolysed red cells and a mixture of cells and serum. Sheep received* 5·0 *c.c. of vaccine s.c. on* 16.8.33; *test* 30.8.33.

Vaccine.	Sheep. Test with culture i.m. (c.c.).		
	1·0	2·0	4·0
H.F.M.B. plus			
(*a*) Sheep serum.................	1 (32951) L	2 (33927) L	3 (33939) L
(*b*) Sheep Hm. cells.............	4 (35007) L	5 (35019) † 1	6 (36770) † 1
(*c*) Sheep Hm. cells and serum..	7 (37241) L	8 (37896) † 2	9 (36932) † 2

Controls: 10 (37509) 0·1 c.c............ † 2
11 (37623) 0·2 c.c............ † 1
12 (35526) 0·3 c.c............ † 1
(† = died. L = lived.)

Result.—The indication given in Table IV is that the addition of sheep serum to medium produces a better vaccine than the addition of sheep haemolysed red cells.

Experiment 5.

As for Experiment 5, but using horse serum and horse haemolysed red cells. Flasks of 500 c.c. capacity were used, the media and anacultures being treated as noted under Experiment I. The inoculation and the test culture were guinea-pig passage strains of *Cl. chauvœi* 64. Sheep received 5·0 c.c. vaccine subcutaneously on 14.9.33 and were tested for immunity on 13.10.33. The test consisted in the intramuscular injection of 1·0 c.c. of culture; all sheep survived (three per group). A control animal receiving 0·1 c.c. of culture lived and another getting 0·25 c.c. died in twenty-four hours. Thus, no comparison can be made of the immunizing value of the three products. (Experiment S. 5148 and sheep 37134, 37157, 37167, 37236, 37255, 37285, 37534, 37546, 37713, 37830, 37882.)

Experiment 6.

The results recorded in Experiments 1 to 5 show that a potent antigen can be prepared in 500 c.c. quantities of horse or ox flesh meat broth plus serum and/or haemolysed red cells of the sheep, ox or horse. However, experimentation showed the difficulty of obtaining serum or haemolysed cells and of adding them to medium under sterile conditions as a routine measure. For this reason, the antigen producing power of Hartley's (1922) and Pope's and Smith's (1932) digest broths was investigated. In addition, the antigen producing power of horse-flesh meat broth plus 5 per cent. horse serum and plus 5 per cent. horse haemolysed cells [these sterilized by incubating them at 37° C. for twenty-four hours in the presence of 0·1 per cent. formalin (40 per cent. formaldehyde)] was ascertained. The media used were as follows:—

Medium 1.—500 c.c. of Hartley's digest broth, meat particles one-sixth to one-fifth by volume; pH adjusted to 7·8; boiled and cooled.

Medium 2.—As medium 1, plus 5 per cent. etherised horse serum.

Medium 3.—500 c.c. of Pope's " straight line " digest broth; meat, etc., as medium 1.

Medium 4.—As medium 3, plus 5 per cent. etherised horse serum.

Medium 5.—500 c.c. of Hartley's digest broth (no meat particles) plus 0·15 per cent. agar. Otherwise treated as medium 1.

Medium 6.—20-litre flask of horse-flesh meat broth (broth about 14 litres, meat particles about one-third by volume). Had been prepared for about one week; not boiled and pH not investigated; 2 per cent. of 0·1 per cent. formolised horse plasma added.

Medium 7.—As medium 6, but plus 5 per cent. of 0·1 per cent. formolised horse haemolysed cells.

Medium 8.—As medium 7.

All flasks were inoculated with a meat broth subculture of a three weeks' old guinea-pig passage culture of strain 64. The subsequent treatment was as noted under Experiment 1.

Order of growth in the various media.—(4 days 37° C.).

Medium 1.—Rather poor, (as judged by opacity and gas production).

Medium 2.—Fair.

Medium 3.—As (1).

Medium 4.—Fair.

Medium 5.—Excellent.

Medium 6.—Excellent, (contaminated with a coccus).

Medium 7.—Poor, (contaminated with a coccus).

Medium 8.—Good, (contaminated with a Gram + aerobe).

The two flasks comprising media 7 and 8 were treated separately, owing to the difference in growth.

Sheep received 5·0 c.c. subcutaneously of the various vaccines on 27.10.33 and were tested for immunity on 16.11.33 (a recent passage culture used). Table V. records the results.

TABLE V.

Immunizing power of vaccines detailed in Experiment 6. Sheep inoculated on 27.10.33; *test* 16.11.33 (Expt. S. 5184).

Vaccine.	Sheep. Test with culture i.m. (c.c.).		
	1·0	2·0	4·0
Medium 1	1 (37143) L	2 (37581) L	3 (37688) L
Medium 2	4 (37716) L	5 (37732) L	—
Medium 3	6 (37767) L	7 (37772) L*	8 (37784) L
Medium 4	9 (37785) L	10 (37812) L	11 (37847) L
Medium 5	12 (37855) L	13 (37876) L	—
Medium 6	14 (37910) L	15 (37911) L	16 (37921) L
Medium 7	17 (37980) L	18 (37990) L	19 (37997) L
Medium 8	20 (38015) L	21 (38017) L	22 (38059) K*

* Very severe reaction. K* Killed 24.11.33 owing to reaction.

Controls: 23 (36907) 0·1 c.c. .... †2
24 (37712) 0·2 c.c. .... †1
25 (37880) 0·4 c.c. .... †1

Result.—The vaccines prepared in all media produced excellent immunity. Even in those flasks in which growth was poor and in which contamination occurred, good antigen was formed.

Experiment 7.

To ascertain if horse-flesh meat broth plus 5 per cent. formolised (0·1 per cent.) horse plasma could be used as a routine medium for the production of vaccine.

To seven 20-litre flasks of horse-flesh meat broth (meat particles about one-third by volume) approximately 5 per cent. formolised horse plasma was added. The media stood for three days at room temperature and were then seeded with a von Hibler culture of a recently passaged strain of 64. The pH of the flasks was not investigated. Incubation was for three days at 37° C. and good growth was obtained in each flask, but a purity test revealed a contamination with a Gram + aerobe. To the Seitzed filtrate, 0·5 per cent. formalin was added and incubation carried on for three days. Sterility tests on the final product passed. Sheep received 5·0 c.c. of vaccine subcutaneously on 7.10.33 and were tested for immunity on 19.10.33 by the intramuscular injection of a twenty-four hours' von Hibler culture. (Experience over many years has shown that the M.L.D. of such a culture is between 0·5 c.c. and 1·0 c.c.)

Result.—The control sheep receiving 1·0 c.c. and 2·0 c.c. of culture died within thirty-six hours. Of the vaccinated sheep, one withstood 2·0 c.c. and another 3·0 c.c. of culture; the third succumbed to the injection of 4·0 c.c.

It is difficult to make a fair comparison between the immunizing power of this vaccine and those produced in smaller volumes of medium, where the inoculum was a meat broth culture. However, the indication is that the degree of immunity was less than that produced by the vaccines made from media and 7 and 8 of Experiment 6, where the inoculum was a meat broth culture. (Experiment S. 5168 and sheep 37683, 37771, 37777, 3804004, and 38021.)

Experiment 7 (*a*).

This was a repetition of Experiment 7 with a few modifications. The pH of the horse-flesh meat broth (20-litres, meat particles one-third by volume) was adjusted to 7·8, 3 per cent. of 0·1 per cent. formolised horse plasma and 0.25 per cent. glucose was added. The inoculum was a meat broth culture of a recently passaged strain of 64. Incubation was for four days at 37° C., the meat was removed and anaculture made by adding 0·4 per cent. formalin and incubating for forty-eight hours.

Sheep received 5·0 c.c. of the vaccine on 9.11.33 and were tested for immunity on 6.12.33, using a meat broth culture of strain 64.

Result.—The control sheep receiving 0·1 c.c. of culture died within forty-eight hours. One immunized sheep which received 1·0 c.c. of culture died, a second which got 2·0 c.c. lived, as did a third into which 4·0 c.c. were injected.

One could conclude that the vaccine prepared as described had a high immunizing value. (Experiment S. 5197 and sheep 35552, 36784, 36879, 36961.)

Experiment 8.

To ascertain the immunizing value of anacultures prepared from one,two and four day cultures.

To a one-litre quantity of donkey-flesh meat broth (meat particles one-third by volume) 3 per cent. horse plasma (formolised 0·1 per cent.) and 0·25 per cent glucose was added, after the medium had been boiled for an hour and the pH adjusted to 7·8. A direct guinea-pig passage culture of strain 64 was used as inoculum. After one, two, and four days' incubation at 37° C., samples of culture were removed, formolised to 0·4 per cent. and incubated for a further forty-eight hours. Sheep received 5·0 c.c. of the one or the other vaccine on 17.11.33 and were tested for immunity on 6.12.33.

Result.—The control sheep were those used in Experiment 7 (*a*). The sheep receiving the one, two, and four day anacultures (3 per anaculture) survived the intramuscular injection of 1·0 c.c., 2·0 c.c. and 4·0 c.c. respectively of the same culture as used for the controls.

Although no answer could be given as to the respective immunizing values of the different vaccines, the conclusion may be drawn that, under certain conditions, excellent immunity is produced in sheep by the use of anacultures made from one, two or four day cultures. (Experiment S 5201 and sheep 35022, 35528, 36814, 36984, 36991, 36961, 37887, 37092, 37408.)

Experiment 9.

To ascertain if there is any difference in the immunizing value of anacultures made in 10-litre quantities of donkey-flesh meat broth, when the inoculum is a meat broth culture of *Cl. chauvœi* and when it is a von Hibler culture.

The pH of two 10-litre flasks of donkey-flesh meat broth (meat particles one-third by volume) was adjusted to 7·8. The inoculum of flasks A and B was as follows: Flask A, guinea-pig liver (killed by i.m. injection of strain 64)➤meat broth➤meat broth➤flask: Flask B, guinea-pig liver (as above)➤meat broth➤von Hibler➤von Hibler➤flask. Incubation was for 3 days at 37° C.: to the meat-particle-free supernatant, 0·4 per cent. formalin was added and incubation carried on for three more days.

Sheep received 5·0 c.c. s.c. of vaccine on 7.3.34 and were tested for immunity on 27.3.34. Table VI. records the results.

TABLE VI (Expt. S. 5117).

To test the immunizing value of anacultures made in donkey-flesh meat broth when the inoculum was a meat broth culture and when it was a von Hibler culture.

Sheep inoculated s.c. on 7.3.34; *test* 27.3.34.

Vaccine.	Sheep. Test with culture i.m. (c.c.).		
	1·0	2·0	4·0
Meat broth inoculum................	1 (36555) L	2 (38846) † 2	3 (37129) L
Von Hibler inoculum................	4 (36996) † 2	5 (33893) L	6 (36680) †o/n

(L = lived ; † = died ; o/n = overnight.)
Controls : 7 (38629) 0·1 c.c............. † o/n
8 (38201) 0·2 c.c............. † 1

Result.—The result given in Table VI. allows of no definite conclusion being drawn. As the von Hibler inoculum vaccine so immunized one sheep that it withstood at least 20 fatal doses of cultures, it would not appear that the inoculum played a very large rôle.

Experiment 10.

To determine the effect on immunity production of altering the pH of the medium, and of the addition of glucose.

Three 20-litre flasks of donkey-flesh meat broth (meat particles one-third by volume) were used. To two, 3 per cent. of horse plasma (0·1 per cent. formolised) was added—the pH of one was left at 6·8 and that of the other brought to 8.

To the third flask of medium at pH 8, 0·25 per cent. glucose was added. The inoculum was a twenty-four hours' old meat broth culture of a recent passage strain of 64. After four days' incubation at 37° C., the meat was removed from each flask, 0·4 per cent. formalin added and incubation continued for a further three days.

Into sheep 5·0 c.c. of vaccine was injected subcutaneously on 2.5.34, the test being carried out on 22.5.34 (intramuscular injection of a twenty-four hours' meat broth culture). Table VII. records the results.

TABLE VII (Expt. S. 5371).

The immunizing power of anacultures made in meat broth at pH 6·8 *and* 8·0 *plus horse plasma and in meat broth, pH* 8 *plus* 0·25 *per cent. glucose.*

Sheep inoculated 2.5.34; *test* 22.5.34.

Vaccine.	Sheep. Test with culture i.m. (c.c.).			
	0·25	1·0	2·0	4·0
M.B. + plasma, pH. 6 8	1 (39493) L	2 (39467) L	3 (39172) L	4 (39307) † 2
M.B. + plasma, pH. 8 0	5 (39398) L	6 (39303) L	7 (39464) L	8 (39398) L*
M.B. + glucose, pH. 8 0	9 (39254) L	10 (39393) L	11 (39160) L	12 (39366) L

Controls. 13 (39953) 0·1 c.c............ † 2
14 (39997) 0 25 c.c........... † 2
(L = lived ; † = died ; * = Sheep very ill, lame, oedema of leg, but recovered.)

Result.—All three vaccines proved to be excellent antigens. The results given in Table VII do not indicate definitely which, if any, was the best.

Experiment 11.

Although excellent vaccine could be made with 20-litre quantities of horse or donkey flesh meat broth containing 0·25 per cent. glucose, a definite practical disadvantage was the inability of this medium to initiate growth with certainty. On several occasions growth either did not occur or was delayed for several days. The addition of serum overcame this difficulty, but one had then to reckon with the possibility of introducing contaminating organisms and in addition, there was the difficulty of obtaining serum in bulk if the method was adopted as routine. One of us had had experience with Colebrooke's liver digest medium* in the cultivation of anaerobes. Excellent growth could be obtained in this medium, without the addition of meat particles, with *Cl. chauvœi*, but a disadvantage was the lowering of the pH that took place with resultant killing of the organisms, and possibly harmful effect upon the antigen produced. To try to overcome this effect, the following modification was tried.

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* See appendix for preparation.

Three parts of ox-liver digest and seven parts of horse-flesh peptone broth (no meat particles) were mixed. Salt was omitted and in its place enough phosphate buffer to make a 0·8 per cent. concentration was added. Seven hundred c.c. at pH 7·4 was boiled for two hours, cooled, inoculated with a recently passaged strain of D and grown for twenty hours at 37° C. Excellent growth resulted, the pH being between 6·5 and 6·7. After adjusting the pH to 7·4, 0·4 per cent. formalin was added and incubation carried on for forty-eight hours.

Sheep received 5·0 c.c. s.c. of the vaccine on 20.9.34 and were tested for immunity on 10.10.34. The method of test was changed. Robertson and Felix (1930) and Henderson (1932) in testing the immunity produced in laboratory animals by the injection of washed boiled suspensions of *Cl. septicum* and *Cl. chauvœi* used spore suspensions activated with calcium chloride. Henderson found that *Cl. chauvœi* could be induced to spore copiously when grown on Dorset's egg medium. Although we never obtained copious sporulation on this medium, we were able, after several attempts, to obtain a suspension rich enough in spores for our purpose. This was heated at 60° C. for half an hour to destroy vegetative elements and then stored at +5° C. It was our intention to use this spore suspension for several months and thus be able to compare the results of several tests. Unfortunately, the suspension lost virulence after only a fortnight, so that comparative work could not be carried out. As activator, adrenalin was employed. We found that, in guinea-pigs, as little as 0·02 c.c. of the 1/1000 dilution received from the manufacturers was sufficient, when diluted to 2·0 c.c., to activate one-tenth of a doubtfully lethal dose of spores. For the sheep test 0·5 c.c. of the spore suspension plus 0·05 c.c. of adrenalin, diluted to 2·0 c.c. with saline, was injected i.m. into four control and the four vaccinated sheep.

Result.—All the control sheep died within forty hours; the vaccinated sheep survived. Although this experiment shows that the vaccine produced in the mixture of digest liver medium and horse-flesh broth was antigenic, we do not know how high the immunity was. From other experiments (not noted in this communication) we have gained the impression that the activated spore test is not a severe one, inasmuch as animals immunized with proved low value antigens (test with culture) survived the injection of the activated spores. (Experiment S. 5484 and sheep 38953, 37626, 41049, 40071, 40771, 40585, 40189 and 40901.)

Experiment 12.

To ascertain if a washed alum precipitate of a *Cl. chauvœi* vaccine produces better immunity than the vaccine itself.

Glenny *et al.* (1926), Glenny and Waddington (1928), and Glenny, Buttle and Stevens (1931) have shown that the alum precipitate of a diphtheria toxoid is a better immunizing agent than the toxoid itself. They attribute this improvement to the decreased solubility of the alum precipitate; it is only slowly absorbed by the body, resulting in increased immunity. One of us (J.H.M.) has had experience of the use of alum precipitate of toxoids of *Cl. welchii*, Type A and B (Wilsdon) and of *Cl. septicum*. The results of immunization with *Cl. welchii* Type B precipitates were disappointing and will be published at an early date. With *Cl. welchii* Type A and with *Cl. septicum*, the definite indication was that alum precipitates were better antigens than the toxoids from which they were prepared.

In Experiment 2, the alum precipitate of a batch of routine medium vaccine (anaculture) proved a better antigen than the anaculture itself. In that instance, the alum was added to the vaccine, and the precipitate washed twice and injected. In this experiment, enough alum was added to make a 1 per cent. concentration, and the precipitate (without being washed) plus the supernatant was injected. As table VIII shows, the experiment is not conclusive inasmuch as the sheep into which the vaccines were injected were not tested at the same time or with the same test culture as were the alum vaccine sheep. However, the immunity of these sheep was so high that, judging from results obtained with other batches made in the same way and about the same time one may justifiably conclude that the alum vaccines were superior antigens. Into two groups of sheep, two batches of vaccines (routine) were injected. One group received batch No. 86 and the other batch No. 87.

To each batch 1 per cent. alum was added and sheep received 5·0 c.c. s.c. The details of the injections and test are recorded in Table VIII.

Result.—As stated above, the experiment allows of no definite conclusions being drawn, but in view of the fact that experience has shown that good value routine vaccines usually produced immunity only to one to four fatal doses of culture (0·3 c.c.–0·5 c.c. of a von Hibler culture was in the region of an M.L.D.), one may be allowed to infer that the alum precipitate was the better vaccine. It will be observed that sheep 11, which received 86 alum precipitate, resisted the inoculation of at least sixty lethal doses of a meat broth culture.

Experiment 13.

To determine if the addition of agar to vaccine would increase the immunizing value.

This experiment was in the nature of a " follow-on " of that detailed above. *Cl. chauvœi*, 64, was grown in 8 × 20 litre flasks of donkey-flesh meat broth (meat particles one-third by volume) plus 0·5 per cent. glucose for three days. Growth was good. The meat was removed and 0·5 per cent. formalin added and incubation carried on for three days. To a quantity of anaculture, enough melted agar was added to give a 0·25 per cent. concentration. Sheep received 5·0 c.c. s.c. of the one or the other vaccine on 11.6.34 and were tested for immunity on 5.7.34 (i.m. injection of a meat-broth culture of strain 64). Table IX records the results.

Table VIII.

Comparison of the immunity by routine vaccines and by alum precipitates.

Vaccine.							
86 (S. 5478)	Sheep	1 (41058)	2 (40414)	3 (40444)	4 (40531)	5 (40538)	6 (40617)
	Dose injected (3/9/34) c.c.	2·0	2·0	5·0	5·0	20·0	20·0
	Test (25/9/34)— (v. Hib. cult.) c.c.	0·5 L	0 5 L	0 5 L	0·5 L	0 5 L	0·5 L
	Controls	7 (40726) 0 5 † 1		8 (40838) 0·5 † 1			
86 alum ppt. (S. 5515)	Sheep	9 (41123)	10 (41038)	11 (41111)			
	Dose injected (25/10/34 c.c.	5·0	5·0	5 0			
	Test (20/11/34)— (M.B. culture) c.c.	2·0 L	4 0 L	6 0 L			
	Controls	12 (41046) 0·1 † 36 hours.					
87 (S. 5485)	Sheep	13 (40654)	14 (40850)	15 (40994)	16 (41010)	17 (41015)	18 (41034)
	Dose injected (18/9/34) c.c.	2·0	2 0	5 0	5 0	20·0	20·0
	Test (9/10/34)— (v. Hib. culture) c.c.	0·5 L	0 5 L	0·5 L	0·5 L	0·5 L	0·5 L
	Controls	19 (40666) 0·5 † 1		20 (41044) 0 5 † 1			
87 alum ppt. (S. 5506)	Sheep	21 (41026)	22 (41124)	27 (41043)	28 (41001)		
	Dose injected (16/10/34) c.c.	5 0	5 0	5 0	5 0		
	Test (6/11/34)— (M.B. culture) c.c.	0·5 L	0 5 L	1 0 L	1 0 L		
	Controls	29 (41022) 0 1 † o n.		30 (41047) 0 25 † 1.			

(† = died; o/n = overnight; 1 = 1 day; hrs. = hours; L = lived; M.B. = horse flesh meat broth culture; v. Hib. cult. = von Hibler culture.)

TABLE IX (Expt. S. 5474).

The immunizing power of anaculture compared with anaculture plus 0·25 per cent. agar. Sheep inoculated on 11.6.34: test 5.7.34.

Vaccine.	Sheep. Test with culture i.m. (c.c.).			
	0·3	0·5	0·5	0·5
Anaculture.............	1 (40174) L*	2 (39944) † 1 0·3	3 (40558) † 1	4 (40520) L*
Anaculture plus agar...	5 (40128) L	6 (40033) L	7 (40157) L	8 (40175) L

Controls: 9 (40131) 0·15 c.c........... † 1
10 (40399) 0·5 c.c............ † o/n

(† = died; L = lived; * = Very ill, lame, leg swollen, recovered.)

Result.—The addition of agar increased the immunizing value of the anaculture to a considerable extent, probably due to decreasing the *in vivo* rate of absorption.

Experiment 14.

To ascertain the immunizing power of washed, boiled *Cl. chauvœi* bacilli. Robertson and Felix (1930) and Henderson (1932, 1933, 1934) have shown that boiled suspensions of *Cl. septicum* and *Cl. chauvœi* are capable, when inoculated subcutaneously into guinea-pigs, of immunizing them against the injection of spores activated with calcium chloride. Henderson suggested that the immunity to *Cl. chauvœi* is produced, in the main, by the "O" somatic antigen of the bacilli. Results, of a confirmatory nature, which will form the subject matter of another communication, have been obtained by one of us (J.H.M.). Roberts (1933) was not able to obtain satisfactory results with Henderson's technique.

The following experiments were carried out to test the method:—

(*a*) Strain 64 was grown in 1 per cent. glucose broth (horse-flesh) plus haemolysed cells and serum for 18 hours. The bacilli wer spun out, washed twice in distilled water, boiled for two hours and enough salt added to make a 0·85 per cent. concentration. About 400 c.c. of medium was required to produce 70 c.c. of a suspension corresponding in opacity to tube 10 of a Burroughs Wellcome nephelometer. Into sheep, 10·0 c.c. of the suspension was injected s.c. on 12.4.33; the test took place on 11.5.33 (the same test as noted in Experiment 1). Table X records the results.

TABLE X.

The immunizing power of boiled bacilli (Expt. S. 5003).
Sheep inoculated on 12.4.33; test 11.5.33.

Sheep.	Test. Culture i.m. (c.c.).	Result.
1 (35333)	1·0	L
2 (35527)	2·0	L
3 (35773)	3·0	L
4 (35783)	4 0	L
Controls.		
5 (35517)	0·5	† 1
6 (35537)	1·0	† 1

(L = lived ; † = died.)

Result.—On the assumption that 0·1 c.c. of the test culture was a lethal dose, it will be seen that boiled bacilli produced a very high degree of immunity.

(*b*) As (*a*), but bacilli obtained from meat broth plus serum. The opacity of the suspension corresponded to tubes 5–6 of the B.W. nephelometer.

These sheep received 5·0 c.c. subcutaneously on 6.3.34. Tested twenty-one days later with culture i.m., the M.L.D. of which was 0·1 c.c. for control sheep, these animals reacted as follows: that receiving 5 M.L.D. lived as did that receiving 20 M.L.D., whilst one which got 10 M.L.D. died. (Experiment S. 5117 and sheep 34043, 37354 and 24543.)

(*c*) A group of six sheep received eight subcutaneous injections of the suspension noted in (*b*); in all, each sheep got 180 c.c. of material, over a period of thirty days. Tested 9 days after the last injection with culture i.m., the following result was got. Two sheep withstood 50, one 100, one 200 and two 300 M.L.D., whilst a non-treated died after receiving one lethal dose (0·1 c.c.). (Experiment S. 5432, sheep 38847, 37889, 37014, 37362, 34130, and 36583.)

(*d*) A suspension prepared as noted under (*b*). The opacity correspond to that of tube 10 of the B.W. nephelometer.

Two sheep which received 5·0 c.c. of suspension s.c. withstood, three weeks later, 5 and 10 M.L.D. respectively of culture i.m. The control sheep died when 1 M.L.D. (0·1 c.c.) was injected. (Experiment S. 5588, sheep 41018 and 39749.)

(*e*) The bacilli contained in 200 c.c. of a twenty-four hours' meat-broth culture were spun out, washed twice in distilled water, resuspended in 200 c.c. of distilled water, boiled for two hours and enough salt added to make a 0·85 per cent. concentration. The opacity was about that of the tube 1 of the B.W. nephelometer.

Three sheep received 5·0 c.c. s.c.; twenty-seven days later, a meat-broth culture was injected i.m. with the following result:—

Control sheep	(39771)	0·1 c.c.	† o/n.
Sheep 1.	(41561)	0·2 c.c.	,,
Sheep 2.	(41535)	0·5 c.c.	,,
Sheep 3.	(41542)	1·0 c.c.	,,

(Experiment S. 5565.)

(*f*) The bacilli from a 24 hours' culture in 25 litres of routine medium were removed. This was accomplished by passing the culture through a paper pulp, washing the pulp in distilled water, squeezing the fluid out of the pulp in a press and finally spinning this material. After two washings, 200 c.c. of a dense suspension was obtained. This was frozen and thawed five times. The freezing chamber of a General Electric Company's refrigerator was used for freezing purposes (−15° C.). This five-times frozen suspension was spun for two hours at 4,000 revolutions per minute, a slightly opalescent fluid being obtained. A portion of it was boiled for two hours, a slight turbidity being produced in the process.

Two groups of sheep were immunized, one with the spun and the other with the spun and boiled supernatant. Table XI records the results.

TABLE XI.

The immunizing power of the supernatant of frozen and thawed bacilli and of this material after boiling for two hours. (Expt. S. 5595.)

Material Injected.	Date and Amount.		Test : culture i.m. (c.c.).	
			13/3/35.	
			0·1	0·25
Supernatant.........	22/2/35, 5·0 c.c.	Sheep...	1 (41523) L	2 (41555) L
Boiled supernatant..	22/2/35, 5·0 c.c.	Sheep...	3 (41532) † 2	4 (37385) † 1
		Controls.	5 (41541) † 1	6 (41520) † o/n
			4/4/35.	
			0·25	0·5
Boiled supernatant..	22/2/35, 5·0 c.c.	Sheep...	7 (38891) L	8 (37397) L
	14/3/35, 5·0 c.c.	Controls.	9 (40679) † 1	—

(† = died; L = lived.)

Results.—In sufficient concentration, the boiled bacillary bodies of *Cl. chauvœi* produced a high degree of immunity in sheep, the test material being a young meat-broth culture, injected intramuscularly. However, when the boiled germs were suspended in an amount of saline equal in volume to that of the culture from which they were obtained, the immunity produced was poor (or absent).

The supernatant of a five-times frozen and thawed dense living suspension was of high antigenicity. The boiling of such a supernatant, whilst reducing greatly the antigenic value, did not entirely destroy the antigen.

The Choice of a Routine Vaccine.

The following points were carefully considered before a decision was made on the medium to be employed in the preparation of vaccine:—

1. The routine medium and method of production had proved satisfactory on past occasions.
2. A potent product could be produced in horse or donkey flesh meat broth plus serum. As before mentioned, there was the difficulty of obtaining serum in quantity and of ensuring its sterile addition to medium. Without serum or haemolysed red cells, there was the definite possibility that growth would not be initiated in all flasks.
3. A tryptic digest muscle medium could be employed or a mixture of Colebrooke's liver digest and horse-flesh infusion broth. These were very seriously considered, but finally rejected owing to the difficulty of preparing a large batch in a working day.
4. Unfortunately Henderson's boiled bacilli vaccine could not be considered owing to the large amount of medium that would be required for its production and to the difficulty that would be experienced in separating the germs from the culture.

Finally it was decided to retain the routine medium, but to introduce some variations. It had been the custom of one of us (J.R.S.) to maintain the *Cl. chauvœi* strain in von Hibler's medium, passaging a culture through a guinea-pig just prior to the inoculation of a batch of medium. Instead, the strain was held in horse-flesh meat broth, care being taken that the pH of the medium was 7·6 just prior to seeding. From a tube, which was a direct culture from the heart blood of a guinea-pig, a subculture was made into von Hibler's medium, at pH 7·6, and after eighteen hours' incubation, flasks of medium were inoculated from this. The von Hibler's medium was used to ensure an inoculum, containing a large number of organisms. The pH of the medium in the flasks (20 litres) was adjusted to between 7·6 and 8·0 just prior to inoculation. Care was taken that the amount of liver particles in the flasks was about one-third of the total volume. After thirty-six hours' incubation at 38° C., when growth and gas formation were vigorous, the liver pieces were removed by sieving, 0·5 per cent. formalin added and incubation carried on for three more days. The final product passed adequate sterility tests and was innocuous for sheep in 20 c.c. amounts subcutaneously.

The results of a year's use of vaccines prepared as just described have been satisfactory.

As an example of the type of vaccine now being prepared after a little more than one year's use of the method just mentioned, the following experiment (15) may be quoted. Six sheep received 5·0 c.c. of vaccine (prepared from 20-litre quantities of medium) subcutaneously and were tested three weeks later by the intramuscular injection of an 18 hours' von Hibler culture. Two withstood 10 M.L.D., two, 20 M.L.D. and two, 40 M.L.D. (Experiment S. 5713 and sheep 43388, 43401, 43417, 43488, 43685, 43934, 43586 and 43111.)

COMMENT.

It is difficult to say if only one change was responsible for the improvement in the vaccine. Whilst retaining the original medium the following alterations have been made:—(1) The maintenance of the *Cl. chauvœi* strain in meat broth instead of von Hibler's medium, (2) the ensuring that the initial pH of all media was 7·6–8·0, (3) a shortening of the incubation time, (4) the leaving out of the re-additions of glucose to the medium and (5) anaculture instead of filtrate used.

The experiments detailed have shown that excellent vaccine may be produced after twenty-four hours' incubation of culture but that no significant difference in immunity production was got with vaccines prepared from media of initial pH 6·8 and 8·0 respectively. However, one may justifiably conclude that better growth could be expected from a medium the pH of which was 8 than from one where it was 6·8. The strain was maintained in meat broth, because experience showed that the virulence did not decrease in this medium whereas it did so in brain medium. Actually after one year's maintenance in meat broth the virulence of strain 64 increased four times. Further, meat broth cultures were usually five to ten times more pathogenic than those from von Hibler's medium.

Although the addition of glucose (with the accompanying adjustment in pH to 7·8) on several occasions, as originally carried out, did lead to the production of a dense growth of the germ with a possible extra formation of antigen, the definite possibility existed that the long incubation period and the metabolic products formed during these " re-growths " might destroy antigen already formed.

Finally, one result that comes out clearly is the consistently good results obtained with small quantities (500 c.c. to 2,000 c.c.) of meat broth medium (Experiments 1, 2, 3, 4, 5 and 8); when 10 to 20 litre volumes were used, satisfactory vaccine was produced but not always of the same high value as with small amounts of medium [Experiments 6, 7 (*a*) and 10 gave good vaccines, and Experiments 7, 9 and 13 vaccines of a lower value]. The probable explanation for this was thought to be the procedure in autoclaving. The small amounts were heated for half an hour at 115° C. whereas to ensure sterility, the large volumes were heated at 115° C. for four hours on two successive days.

An experiment (16) set up to investigate this question did not yield absolutely satisfactory results. The batch of medium used was noted under "choice of a routine vaccine" (Experiment 15). Flasks of 500 c.c. capacity were used—one was autoclaved twice (one day interval) at 110° C. for one hour and the other at 115° C. (on two occasions) for four hours. The vaccines (anacultures) prepared from these media were injected subcutaneously in 5·0 c.c amounts into two groups of four sheep each. The test was carried out along with that conducted for Experiment 15 with the following results:—

> Vaccine from flask sterilized for 2 x 1 hour at 110° C.—number of fatal doses withstood by sheep—(1) 5, (2) 10, (3) 20, (4) <40.
>
> Vaccine from flask sterilized for 2 x 4 hours at 115° C—number of fatal doses withstood by sheep—(1) <5, (6) 10, (7) <20, (8) <40.

It should be noted that the same medium sterilized in bulk (Experiment 15) produced excellent vaccine even although subjected to 2 x 4 hour periods at 115° C. This apparently is at variance with the results noted, but since over-sterilization is detrimental to medium (shown up when small quantities are used) one can conclude that the same detrimental effect occurs when large volumes are employed. However, in this case, all the medium is not subjected all the time to the high temperature and therefore the sterilization of 500 and 20,000 c.c. quantities is not to be compared.

Although it is possible and even probable that over-sterilization played a part in the production of low value vaccines, it is obvious that it is not the whole explanation. Experiments destined to clear up this point are at present under consideration.

CONCLUSIONS.

1. Potent *Cl. chauvœi* vaccine (anaculture) may be made in horse-flesh infusion broth plus 1·0 per cent. peptone, 0·85 per cent. salt and 2—5 per cent. of serum and/or the haemolysed red cells of the horse, ox and sheep (meat particles one-third by volume).

2. Tryptic digests of horse muscle media produce satisfactory vaccines.

3. Boiled, dense suspensions of *Cl. chauvœi* injected subcutaneously into sheep produce a high degree of protection to culture injected intramuscularly.

4. The medium and method finally decided on as a routine measure was: ox-liver infusion broth (liver particles one-third by volume) plus 1·0 per cent. peptone, 0·85 per cent. salt and 1 per cent. glucose added after sterilization, pH 7·8, 36 hours' incubation, and 0·5 per cent. formolization for three days at 37° C.

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APPENDIX.

PREPARATION OF ROUTINE MEDIUM.

Boil one part of minced ox liver with two parts of tap-water for half an hour.

Fill into flasks of 20 litres capacity and add 1 per cent. peptone, 0·3 per cent. sodium chloride and 0·2 per cent. dibasic sodium phosphate (Na_2HPO_4).

Sterilize at 115° C. for 4 hours on each of two successive days.

Add enough of a saturated watery solution of glucose (sterilized at 110° C. for 1 hour on each of two successive days) to make a 1 per cent. solution and adjust pH to 8·4.

Incubate to test sterility.

PREPARATION OF ROUTINE VACCINE.

Inoculate flask with 50-100 c.c. of a 20 hours' von Hibler culture and incubate at between 37° C. and 39° C. After 3-4 days, gas formation having almost ceased, the pH is readjusted to 8·4 and enough sterile saturated glucose solution is added to make a 1 per cent. concentration. Vigorous gas formation again occurs. The addition of glucose and the readjustment of the pH is repeated on one or two further occasions at 3-4 days interval. The culture is clarified by passing it through paper pulp and sterilized by filtration through a E. K. Seitz filter.

To the sterile filtrate enough formalin is added to make 0·4 per cent. concentration and incubated for 4-6 days.

PREPARATION OF MEAT BROTH. (Volumes of 10-2,000 c.c.)

To one pound of finely minced horse muscle add one litre of 0·5 per cent. saline solution.

Boil for one hour and filter.

Neutralize and put in cold room over-night.

Refilter.

Add 1 per cent. Witte peptone.

Adjust pH to 8·5.

Boil and filter.

Fill into tubes or flasks containing enough washed cooked minced meat to make one-third by volume.

Autoclave for half an hour at 15 pounds pressure.

In the case of 10-20 litre quantities autoclave for 4 hours on each of two successive days.

The washed meat is obtained by adding to distilled water the minced meat from which the extract was made. The mixture is brought to the boil and strained through muslin. The meat is then pressed until no more fluid exudes.

PREPARATION OF TRYPTIC DIGEST LIVER BROTH.

Mince up ox liver and to every pound add one litre of water. Heat to 45° C. and add 1 per cent. trypsin and make alkaline. Keep at 45°-50° C. and allow to digest for 1½ hours. Then make acid with acetic acid and boil for 5-10 minutes. Filter, make alkaline and autoclave for half an hour at 15 pounds pressure. Filter through paper, tube and re-autoclave.

Section IV.

Plant Studies
AND
Poisonous Plants.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.

By DOUW G. STEYN, Section of Pharmacology and Toxicology, Onderstepoort

(Continued from *Onderstepoort Journal of Veterinary Science and Animal Industry*, Vol. 4, No. 2, 1935.)

COMPOSITAE.

Denekia capensis Thunb. (O.P.H. No. 8895; 23.7.34 N.H. No. 16485).

Common name: ——

Origin: Syferkraal, P.O. Potgietersrust, Transvaal.

State and stage of development: Dry and in the late flowering and seeding stage.

Sheep 38899 (*full mouth*, 29 *Kg.*) received 200 gm. of dry plant daily, except Sundays, for a period of twenty-five days. Total amount of dry plant drenched = 4,200 gm.

Sheep 34170 (*full mouth*, 31 *Kg.*) received 400 gm. of dry plant daily, except Sundays, for a period of twenty-five days. Total amount of dry plant drenched = 8,400 gm.

Result: Negative.

Geigeria aspera Harv. O.P.H. No. 12844; 16.10.34).

Common name: Vomiting-bush; vermeerbossie.

Origin: Skaapplaats, P.O. Wolvehoek, O.F.S.

State and stage of development: Fresh and in the pre-flowering stage. (*N.B.*—New growths on plants of previous season.)

Sheep 38193 (4 *tooth*; 35 *Kg.*): 600 gm. of the fresh plant at 3.30 p.m. on 16.10.34.

17.10.34—9 a.m.—hoven, apathetic, accelerated pulse and laboured respiration. Received another 600 gm. of fresh plant. Vomiting occurred about three hours ofter administration of the second dose. Temperature 104·8° F.

18.10.34—8 a.m.—animal found dead.

Post-mortem appearance.—Pronounced general cyanosis; rumen markedly distended with gas; hyperaemia of ruminal wall; acute catarrhal enteritis; pronounced hyperaemia of the lungs; haemorrhages in the mucosa of the thoracic portion of the trachea.

The plant was then dried in the shade and administered to a sheep to ascertain whether it had decreased in toxicity.

Sheep 38945 (4 *tooth; 30 Kg.*): 400 gm. dry plant on 20.11.34.

21.11.34—appears ill; laboured respiration; apathy; hoven. Received another 400 gm. dry plant.

22.11.34—do. Received another 400 gm. dry plant.

23.11.34—do. Lips stained with ruminal contents. Received another 400 gm. dry plant.

24.11.34—do. Temperature 104·8° F. Received another 400 gm. dry plant.

26.11.34—do., vomiting; temperature 104·6° F. Received 200 gm. dry plant.

17.11.34—do., vomiting. Drenching discontinued.

28.11.34—do.; improving; temperature 104° F.

29.11.34—improving; temperature 104° F.

30.11.34—improving; temperature 104·4° F.

1.12.34—improving; temperature 102·4° F.

2.12.34—apparently normal.

Forty grams of dry plant is equivalent to about 100 grams ot fresh plant.

Two thousand six hundred gm. *dry plant* (equivalent to about 6,500 gm. fresh plant(only caused transitory symptoms of *vermeersiekte* in sheep 38945, whilst 1,200 gm. fresh plant (equivalent to 480 gm. dry plant) caused death in sheep 38193.

It therefore appears that the fresh plant decreases in toxicity during the process of desiccation. The result of this experiment is however not conclusive proof that such is the case as owing to the fact that a limited amount of plant material was available only two sheep could be employed in the test.

Inula graveolens Desf. (O.P.H. No. 1792; 12.6.35).

Common names: Khaki bush, Khaki weed, stinkweed (Australia), kakiebos.

Origin: Stellenbosch, Cape Province.

State and stage of development: Dry and in seeding and post seeding stage.

The person from whom the plant was received stated that she had used it with great success in the treatment of a case of rheumatism after other recognised cures had failed to give relief.

In Australia it is used in the treatment of asthma and in Europe it is administered in colic, dysuria and amenorrhoea. A volatile oil with a greenish fluorescence has been isolated by Schimmel and Co. and probably contains *bornyl acetate*. According to Merck the plant apparently contains two active principles, which have a paralysing effect on the central nervous system (Watt and Breyer-Brandwyk, 1932).

Rabbit A (2·4 *Kg.*) received 10 gm. dry plant twice daily for seven days, that is a total of 140 gm. in the course of seven days.

Rabbit B (2·25 *Kg.*), ditto.

Sheep 43448 (35 *Kg.*) received 300 gm. dry plant daily on five consecutive days, that is a total of 1,500 gm. dry plant.

Result: Negative.

Pyrethrum extract.

The extract is obtained from the flowers of *Pyrethrum carneum, P. caucasium, P. cineriafolium* and *P. roseum.*

In the course of experiments conducted with locust poisons the effects of *Pyrethrum* extracts upon locusts were investigated. Extracts of *Pyrethrum* flowers are very poisonous to cold-blooded animals but much less so to warm-blooded animals, owing to the fact that the *pyrethrines* are readily saponified and broken down in the system of the latter animals (Magens, 1932).

It is a well-known fact that the *pyrethrins* are not very toxic to stock. The "Pyrethrin extract" used in this experiment contained approximately 50 per cent. pure *pyrethrin* and for the remaining "essential oils" of the plant.

Sheep 40657 (2-*tooth*, 22 *Kg.*) received 50 c.c. of the extract per os at 9 a.m. on 5.9.34.

6.9.34—apparently healthy.

7.9.34—do.

8.9.34—do.

9.9.34—listless, laboured respiration and accelerated and strong pulse.

10.9.34—8 a.m.—very listless, staggering gait, marked ataxy and weakness in hind-legs, pronounced dyspnoea. Eventually the animal was unable to rise (general paralysis). It died at 9 a.m. with symptoms of asphyxia and with the head drawn backwards.

Post-mortem appearances (interim about 1 hour).—Blood very watery and stained the finger badly; oedema of the bronchial and mediastinal lymphglands; pronounced hyperaemia and oedema of the mucous membrane of the posterior portion of the ruminal and reticulum wall; acute catarrhal enteritis affecting the jejunum and ileum.

Senecio bupleuroides DC. (O.P.H. No. 11490; 26.9.34; N.H. No. 16642).

Common name: Ragwort.

Origin: Allerton Laboratory, Pietermaritzburg, Natal.

State and stage of development: Wilted and in the flowering stage.

Sheep 34170 (*full mouth,* 42 *Kg.*) received 1,200 gm. of the dry plant on three consecutive days (equivalent to about 3,500 gm. of fresh plant).

Result: The animal developed no symptoms of poisoning except transient fever.

Senecio (nearest *Senecio Burchellii* D.C.) (O.P.H. No. 15603; 23.11.34).

Common name: Ragwort.

Origin: Boksburg, Transvaal.

State and stage of development: Fresh and in the flowering stage.

Sheep 38899 (4 *tooth;* 31 *Kg.*) received 2,000 gm. of the fresh plant and 1,000 gm. of the dry plant in the course of ten days.

Result: Negative.

GRAMINEAE.

Sorghum saccharatum Pers (O.P.H. No. 524; 15.4.35).

Common name: Sweet sorghum, black amber cane, soetriet.

Origin: Newcastle, Natal.

State and stage of development: Fresh and in the late seeding stage.

The fresh leaves were found to contain approximately 0·15 per cent. hydrocyanic acid.

Acacia giraffae Willd. (O.P.H. No. 1230; 8.5.35).

Common names: Transvaal camelthorn, Transvaalse kameeldoring.

Origin: Colorado Ranch, Rustenburg, Transvaal.

State and stage of development: Dry mature pods.

The Manager of the Colorado Ranch informed the writer that severe losses amongst cattle had occurred in a camp where camelthorn trees are growing abundantly. These trees had never borne and shed so many pods in previous years as during this season. Another fact that should be mentioned is that at the time the losses occurred grazing was very poor in the camp concerned.

The Manager stated that the animals were usually found dead, no symptoms having been noticed prior to death. Autopsy revealed no characteristic lesions, the only striking feature being that in all cases the rumen contained large numbers of partly masticated camelthorn pods. Unfortunately no specimens of ruminal contents were submitted for analysis.

The pods were found to contain approximately 0·08 per cent. hydrocyanic acid.

For further information concerning the toxicity of this tree see Steyn and Rimington, 1935.

Acacia saligna Wendl. (O.P.H. No. 750; 25.4.35).

Common names: Port Jackson willow, golden willow.

Origin: Baakens River Native Reserve, Port Elizabeth.

State and stage of development: Fresh young shoots with no flowers or fruits.

Sheep 42538 (4 *tooth*; 32 *Kg.*) received 1,200 gm. of fresh leaves on two consecutive days.

Result: Negative.

Hydrocyanic acid test.

(1) Fresh leaves alone: negative.
(2) Fresh leaves alone + chloroform: negative.
(3) Fresh leaves alone + emulsion: negative.

These results were confirmed by tests conducted by Dr. C. Rimington.

Cassia absus L. (O.P.H. No. 2790; 11.5.34: N.H. No. 16430).

Common name: ....

Origin: Potgietersrust District, Transvaal.

State and stage of development: Dry and in the flowering and seeding stage.

Sheep 38885 (6 *tooth; 25 Kg.*) received 1,000 gm. of the entire dry plant in the course of five days.

Result: Negative.

Crotalaria Burkeana Benth. (O.P.H. No. 5774; 30.5.34).

Common name: Stiff-sickness bush; stywesiektebos.

Origin: Matibaskraal, P.O. Rustfontein School, Pietersburg District.

State and stage of development: Dry and in the late seeding stage.

The stems, leaves and pods were ground and drenched to sheep.

Sheep 38945 (6-*tooth; 28 Kg.*) received 100 gm. dry plant on 6.7.34.

7.7.34—appears healthy. 100 gm. dry plant.

8.7.34—do. Not drenched.

9.7.34—slight painfulness in front hoofs. 100 gm. dry plant.

10.7.34—slight painfulness in front hoofs. 100 gm. dry plant.

11.7.34—slight painfulness in front hoofs. 100 gm. dry plant.

12.7.34—appears normal again. 100 gm. dry plant.

13.7.34—drenching discontinued.

Sheep 38193 (6-*tooth; 29 Kg.*) received 400 gm. dry plant on 6.7.34.

7.7.34—8 a.m.—peculiar gait as if all feet are painful. The hind-legs are placed well under the body and back arched. All hoofs, especially those of the hind-legs, painful and warm; temperature 103·8° F. Another 400 gm. of dry plant; temperature 103·8° F.

8.7.34—condition slightly better; not drenched.

9.7.34—do.; 400 gm. dry plant.

10.7.34—condition almost normal; 400 gm. dry plant.

11.7.34—do.; 400 gm. dry plant.

12.7.34—apparently healthy; 400 gm. dry plant.

13.7.34—do.; drenching discontinued.

Result: Sheep 38193 definitely exhibited symptoms of an acute laminitis, which surprisingly passed off in spite of continued drenching of the animal. The animals were kept under observation for four months, but no elongation of the hoof was noticed.

As soon as fresh plant material is available this experiment will be continued.

Lotononis calycina var. hirsutissima Düm. (O.P.H. No. 464; 13.4.35: Cryptogamic Herb No. 3459).

Common name:———.

Origin: Tarlton Station, Krugersdorp District (Collected by Mr. L. C. C. Liebenberg of the Division of Plant Industry).

State and stage of development: Dry and in the flowering and seeding stage. The plant is affected by fungi.

Hydrocyanic acid test.

(1) 1 gm. of entire plant—negative.

(2) 1 gm. of entire plant + chloroform—negative.

(3) 1 gm. of entire plant + emulsion—negative.

LILIACEAE.

Dipcadi glaucum Bkr. (O.P.H. No. 22784; 28.3.35).

Common name: Wild onion, wilde-ui, malkop-ui.

Origin: "Rusgenoeg", Louis Trichardt District.

State and stage of development: Fresh bulbs in the post seeding stage.

Fresh leaves.

Rabbit A (1·85 *Kg.*): 10 gm. at 11 a.m., 28.3.35.
Rabbit B (1·9 *Kg.*): 30 gm. at 11 a.m., 28.3.35.
29.3.35—appears healthy. 15 gm. fresh leaves.
Result: Negative.

Fresh bulbs.

Rabbit A (2·2 *Kg.*): 10 gm. at 11.15 a.m., 28.3.35.
Result: Negative.
Rabbit B (2·35 *Kg.*): 30 gm. at 11.15 a.m., 28.3.35.
29.3.35—appears healthy. 35 gm. fresh bulbs.
30.3.35—diarrhoea, not feeding.
31.3.35—diarrhoea, improving.
1.4.35—apparently healthy.
2.4.35—apparently healthy.

On a number of previous occasions the plant has been proved toxic to sheep and rabbits. The decreased toxicity of the above specimen is probably due to its being tested in the post seeding stage.

Material (O.P.H. No. 15130; 12.11.34) of this plant in the flowering stage collected on the Research Station, P.O. Mara, Louis Trichardt District, was drenched to a sheep.

Sheep 40623 (6-*tooth;* 31 *Kg.*): 800 gm. of the fresh bulbs, leaves and flowers at 4 p.m. on 12.11.34.

13.11.34—not feeding well, listless, another 800 gm.

14.11.34—7.30 a.m.—standing in a corner of the pen pressing hard against the fence; temperature 106·4° F.; profuse diarrhoea (faeces yellow and watery); very apathetic; not feeding; groaning; dyspnoea; accelerated and strong pulse (12·6 p.m.)

15.11.34—7.30 a.m.—Died previous night.

Post-mortem appearances.—Interim approximately five hours. Rumen markedly distended with gas; intense general cyanosis; acute catarrhal abomaso-enteritis.

Ornithogalum Pretoriense Bkr. (O.P.H. No. 18715; 8.1.35).

Common names:———.

Origin: Queenstown District, Cape Province.

State and stage of development: Fresh and in the flowering stage.

Rabbit (2·1 *Kg.*): 50 gm. of the fresh bulb and leaves per os on 8.1.35.

Result: Negative.

Scilla (=Mogg 5630 named *Scilla ovalifolia* Bkr) O.P.H. No. 12506 A; 5.10.34; N.H. No. 16646).

Common names: ———.

Origin: "Rietpoort", P.O. Sandspruit, Eastern Transvaal.

State and stage of development: Fresh and in the flowering stage.

Rabbit A (1·8 *Kg.*): 10 gm. fresh bulbs, leaves and flowers on 5.10.35.

6.10.35—apparently healthy; 10 gm.

Rabbit B (2 *Kg.*): 20 gm. fresh bulbs, leaves and flowers on 5.10.35.

6.10.35—apparently healthy; 20 gm.

Result: Negative.

Tulbaghia acutilobia ex descrip. (O.P.H. No. 12506 B; 5.10.34. N.H. 16647).

Common name:———.

Origin: "Rietpoort", P.O. Sandspruit, Eastern Transvaal.

State and stage of development: Fresh bulbs in the seeding stage.

Rabbit A (1·8 *Kg.*): 10 gm. fresh bulbs on 5.10.34.

6.10.34—apparently healthy; another 10 gm.

7.10.34—do.

8.10.34—do.

9.10.34—do.

Rabbit B (2 *Kg.*): 20 gm. fresh bulbs on 5.10.34.

6.10.34—apparently healthy; another 20 gm.

7.10.34 to 10.10.34—apparently healthy.

Result: Negative.

Urginea altissima Bkr. (O.P.H. No. 15708; 23.11.34.)

Common name: Maerman.

Origin: " Bierkraal ", Rustenburg District.

State and stage of development: Fresh bulbs with flowers (flower-stalks 3-4 feet long).

Rabbit A (1·8 *Kg.*): 10 gm. juice expressed from fresh bulbs on 23.11.34.

......*Result:* Negative.

Rabbit B (2·0 *Kg.*): 60 gm. juice expressed from fresh bulbs on 23.11.34.

23.11.34—not feeding.

24.11.34—not feeding.

25.11.34—apparently healthy.

Rabbit C (2·1 *Kg.*): 120 gm. juice expressed from fresh bulbs on 23.11.34.

24.11.34—7.30 a.m.—found paralysed in cage; respirations slow and deep; heart-beat 30 p.m. irregular and weak.

25.11.34—8 a.m.—still paralysed; killed *in extremis*.

Post-mortem appearance.—Hyperaemia of the lungs; dilatation of both heart ventricles; hyperaemia of the kidneys; pronounced hyperaemia of the mucosa of the stomach and small intestine.

OXALIDACEAE.

Oxalis Smithii Sond. (O.P.H. No. 1897; 11.6.35).

Common names:———.

Origin: Port Elizabeth.

State and stage of development: Fresh bulbs with no flowers or seed.

Rabbit A (2·8 *Kg.*): 20 gm. fresh bulbs daily for three days.

Rabbit B (2·3 *Kg.*): 20 gm. fresh bulbs daily for three days.

Result: Negative.

SANTALACEAE.

Thesium triflorum Thb. (O.P.H. No. 8637; 16.7.34: N.H. No. 16478).

Common name: Gifbossie.

Origin: " Springfield ", P.O. Tafelberg, Cape Province.

State and stage of development: Dry plant in seeding stage.

Rabbit A (2·1 *Kg.*): 10 gm. of dry plant daily for four days.

Rabbit B (2·2 *Kg.*): 20 gm. of dry plant daily for four days.

Result: Negative.

VITACEAE.

Cissus Juttae.

Common names: Wild grape, wilde druiwe.

Origin: "Auros", P.O. Otavi, South West Africa.

State and stage of development: Dry leaves submitted. Specific name of plant was sent by the person who submitted it.

Sheep 38945 (*full mouth*; 31 *Kg.*) received 3,200 gm. of the dry leaves in the course of nine days.

Result: Negative.

ACKNOWLEDGMENTS.

I am indebted to Dr. E. P. Phillips, Principal Botanist, Division of Plant Industry, Pretoria, and to Mr. A. O. D. Mogg, Mr. Liebenberg and Miss Verdoorn, botanists in the Division of Plant Industry, for the identification of plant specimens; to Mr. M. G. van Niekerk I am indebted for assistance rendered during the course of the experiments.

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Section V.

Mineral Metabolism.

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Studies in Mineral Metabolism XXXIV.

The Effect of Sulphur upon the Weight and Wool Production of Sheep when Food Intake is not Limited.

By P. J. DU TOIT, A. I. MALAN, J. W. GROENEWALD and M. L. BOTHA, Sections of Biochemistry, Nutrition and Wool, Onderstepoort.

IN a recent article by du Toit *et al* (1935) it was concluded that a daily dose of 5 grms. of sulphur given over a period of twelve months was without significant effect on sheep kept on a productive ration but a limited intake. It follows, therefore, that the sulphur fed was without visible effect on the digestion and utilization of the food given, for neither the weight of nor the wool produced by the sulphur-fed group showed significant differences from that of the control group. It is obvious, however, that the experiment concerned would not have indicated increased food consumption and subsequent increased body weights and wool production should the feeding of sulphur to sheep stimulate appetite. An investigation was therefore begun in May, 1934, in which sulphur was given to sheep under conditions which did not limit the amount of food consumed but merely registered the individual intakes periodically.

EXPERIMENTAL DETAILS.

Twenty uniform twelve-months-old Merino ewes, divided by randomization into two groups of ten each were used in the experiment. As in the previous investigation the sheep were fed in individual feeding boxes daily from about 2 p.m. until 8.30 a.m. the following morning. All the food except greenfeed was given in two feeding-boxes, one for the hay and one for the maize. The amount of food given was such that some of it, i.e. both hay and maize, was left over. This procedure ensured maximum food consumption of each individual animal. The food left over was weighed back at intervals which were determined naturally by the poorest eaters whose feeding boxes showed the fastest accumulation of food. Whenever food was weighed back all the boxes were emptied even those in which only a small accumulation of food had taken place.

Greenfeed when given in the individual feeding pens was not taken well, probably partly due to the abundance of food present which necessitated the accumulation of some greenfeed, creating therefore, excellent conditions for deterioration. The sheep showed little inclination to take more than very small quantities of the greenfeed. Hence, shortly after the beginning of the experiment, the sheep were given greenfeed in a common trough replenished daily and to which they had free access when not in the individual feeding pens. This trough was placed in the paddock alongside that in which the feeding pens were built, and was protected from rain and sunshine. Under these conditions the consumption of greenfeed was found to be satisfactory and the practice was continued throughout the course of the experiment.

No special arrangements were made for exercising the sheep, but there was every reason to believe that the daily routine of the experiment, such as letting the sheep into and out of the feeding-pens, daily inspection in the common pen, driving the sheep to the weighbridge at intervals and incidental handling of the animals, provided sufficient exercise. Furthermore, the animals were run in a common pen when they were not in the feeding boxes. The size of this pen (25 by 12 yards) where, incidentally, the sheep always had access to drinking water, allowed the sheep to move about freely which also provided some exercise.

The animals were weighed at monthly intervals, individual food consumption was recorded, wool growth for the full period was registered, 5 grms. of flowers of sulphur made into a paste with water was given daily in a spoon to each animal in one group of ten animals while the remaining animals were kept as controls. The experimental period lasted from 28.5.34 until 27.6.35, when the dosing of sulphur was stopped but both groups kept under the experimental conditions for another four months. The sheep were tested periodically for the presence of intestinal worms and were always found to be practically free.

Experimental Results and Discussion.

Weights.

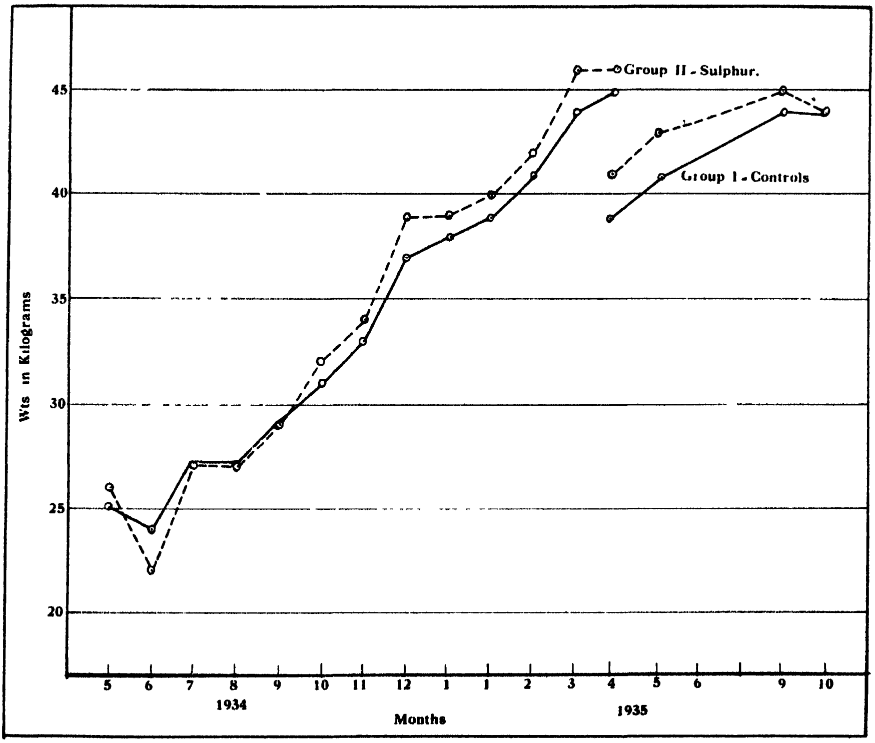
The average weight-curves of the respective groups of sheep are given in the figure below and the individual weights are given in Table I.

The weight curves given in Figure 1 exclude the possibility of a significant difference in weight between the group of sheep receiving a supplement of sulphur daily and the control group. The greatest average difference in weight between the two groups in the course of the experiment was 2 kgs., which was reduced to 0·3 kg. during the post experimental period. From the individual weights given in Table I it will be seen that apparently not a single sheep in the sulphur fed group reacted to sulphur feeding. As a matter of fact the heaviest sheep at the close of the experiment belonged to the control group and this animal was the heaviest throughout the latter half of the experiment. The sudden drop in weight during May, 1935, was due to shearing and is of course shown in both curves. All the sheep in the experiment were in excellent condition throughout the course of the investigation as would be anticipated from a study of the weight curves and attained the really remarkable average weight for sheep of this type of approximately 100 lb. at two years of age.

Food Consumption.

As already stated food consumption was registered by weighing back periodically the food left over in the feeding boxes. Crushed yellow maize was fed in the one box and lucerne hay in the other while the greenfeed was given in a trough in the common paddock. The amounts of both maize and Hay given were such that some was invariably left over daily by practically all the sheep while the greenfeed was not consumed totally on a single occasion.

Fig. 1. -Average weight curves.



The amount of crushed maize given per sheep per day varied from 450 grms. at the beginning of the experiment to 500 and 600 grms. at later stages, with an average daily consumption of 500 grms. per day in group 1 for the entire period and 520 grms. in group 2. Likewise the hay consumptions per head per day were 160 grms. and 165 grms. respectively for groups 1 and 2. The low consumption of hay is naturally due to the abundance of concentrate given and taken. It would appear therefore that group 2, i.e. the group receiving a daily supplement of sulphur, ate slightly more

Table I: Group I: Controls.
Weights in Kg.

D.O.B.	1934.								1935.									
	21/5	22/6	24/7	20/8	21/9	26/10	26/11	31/12	4/1	23/1	23/2	22/3	23/4	28/5	28/5	27/6	28/9	28/10
40251............	23·6	23·1	27·2	28·5	26·7	30·3	28·5	31·2	33·0	33·9	35·3	36·2	39·3	40·7	35·7	38·0	39·8	36·6
40252............	25·3	22·7	25·8	25·8	26·3	29·4	29·4	36·2	35·7	33 0	36·6	36·2	41·1	41·6	33·9	38·4	38·9	38·4
40253............	26·7	25·3	28·5	29·4	30·3	33·9	36·2	40 7	41 6	40 7	42 0	45·8	47·6	51·3	45·4	45·8	49·4	50·0
40336............	23·6	22·7	25·8	26·3	30·8	29·9	33·5	37·1	37·5	35·7	18·4	39 8	44·3	45 4	40 2	41·6	45·4	45·4
40255............	21·3	18·6	21·8	23·1	24·0	25·8	27·6	30·8	32·1	31·2	32·6	34·8	38·0	38·4	32·6	34·8	36·6	38·0
40256............	24·9	22·7	26·7	27·6	29·4	30·3	32·1	34·4	35·3	34 4	34 4	36·2	37·1	39·3	33·5	35·7	38·4	38·0
40257............	31·7	29·5	30·8	28·1	31·2	33·0	35·3	36·6	38·0	38·4	40·7	42·0	45 4	45·4	39·3	38·9	40·2	43·4
40258............	24·5	24·5	27·6	28·1	30·8	31·7	34 8	38 4	39·8	39·3	42·0	43·8	47·2	50·0	43·8	44 7	47·6	48·1
40259............	23·6	23·1	26·3	25·4	28·5	31·7	32·6	38·4	39·3	37·5	38·0	41·1	43·8	42·9	37·1	41·6	43·4	45·4
40260............	28·5	23·6	29·9	29·0	33·9	36·2	38·4	42 9	45 4	44 3	46·7	50 9	56·7	56 7	51·8	52·7	55·4	57·5
Totals.......	253·7	235·8	270·4	271·3	291·9	312·2	328·4	366·7	377·7	368·4	386·7	406 8	440·5	451·7	393·3	412·2	435·1	440·—
	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Averages....	25·37	23·58	27·04	27·13	29·19	31·22	32·8	36·67	37·77	36 8	38·67	40·68	44·05	45·17	39·3	41·22	43·51	44·05

TABLE I: GROUP II.

Weights in Kgs. 5 grms. S. per day.

D.O.B.	1934.								1935.									
	21/5	22/6	24/7	20/8	21/9	26/10	26/11	31/12	4/1	23/1	23/2	22/3	23/4	28/5	28/5	27/6	28/9	28/10
40261............	23·6	19·0	20·9	23·1	26·7	28·5	29·0	34·4	35·3	33·9	34·8	37·1	40·7	41·1	34·4	36·6	40·7	38·0
40262............	28·1	20·4	26·7	26·3	27·2	31·7	32·1	38·9	41·1	39·3	42·5	46·7	50·0	50·9	45·4	47·2	43·8	45·4
40263............	23·1	21·3	22·7	23·1	23·6	18·6	—	—	—	—	—	—	—	—	—	—	—	—
40343............	29·9	29·0	32·1	30·8	34·8	37·1	38·9	41·6	42·0	41·1	43·8	45·8	48·5	48·5	44·3	45·4	46·3	47·2
40265............	29·5	26·7	30·8	30·8	34·4	36·6	37·5	41·6	43·4	43·8	45·8	46·7	52·2	50·9	45·4	49·0	46·7	45·4
40266............	25·3	20·4	25·8	25·4	27·2	31·7	32·6	41·6	37·1	37·1	38·4	39·3	42·5	41·1	35·7	39·8	40·2	41·1
40254............	27·2	23·1	28·5	29·0	29·4	32·1	33·0	37·1	35·3	35·3	36·2	38·0	40·7	41·1	35·3	38·0	40·7	42·5
40268............	25·8	19·5	24·0	25·8	27·6	31·7	33·5	33·9	38·9	37·5	38·4	40·7	44·7	39·8	45·4	45·4	45·4	45·4
40269............	24·0	23·1	27·6	27·2	29·4	33·9	33·9	38·0	39·3	38·4	38·4	41·1	44·3	45·8	40·2	43·4	44·3	45·8
40333............	27·2	19·9	28·1	29·4	31·7	34·8	36·2	39·8	40·2	40·2	42·9	46·3	47·6	50·9	45·4	45·8	—	48·5
TOTALS.......	263·7	222·4	267·2	270·9	292·0	316·7	306·7	346·9	352·6	346·6	361·2	381·7	411·2	415·0	365·9	390·6	348·1	399·5
	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AVERAGES....	26·37	22·24	26·7	27·09	29·2	31·67	34·07	38·5	39·2	38·5	40·1	42·4	45·7	46·1	40·6	43·4	44·8	44·4

food than the control group. However, on submitting the data on food-consumption to statistical analysis it was found that the difference in intake between the two groups was quite insignificant. It must be concluded therefore that 5 grms. of sulphur given daily, except Sundays, to the sheep did not effect the appetite of the animals significantly and furthermore, that the utilization of food was unaffected by the sulphur in sofar as weight increase of the animals could be used as a criterion of food utilization. The sheep consumed on an average about one pound of greenfeed daily.

It should be mentioned that the sulphur was dosed in the form of a paste. Fifty grms. of sulphur were weighed out, transferred to a screw-topped fruit-jar, enough water added to form a paste with the sulphur, the jar closed and the contents shaken vigorously. This method of preparing the paste was very successful, whereas it was exceedingly difficult to moisten the sulphur particles by stirring or gentle shaking in an open container. Dosing the dry powder was not found to be practicable for routine purposes over periods, as the animals easily inhaled the powder, while administering the sulphur through a stomach tube took too long. The paste was dosed in a spoon and with a little practice it was quite easy to transfer ten approximately equal quantities of paste from the common container holding 50 grms. to the animals' mouths.

Wool Production.

The sheep were shorn twelve months after the beginning of the experiment. The weights of the grease wool (unskirted) are given in Table II.

TABLE II.

Weight of Wool given in Kg.

Nos. of Animals.	Control Group. (Kg. wool.)	Nos. of Animals.	Group receiving 5 gm. S. daily. (Kg. wool.)
40251	4·4	40261	5·3
40252	4 9	40262	5·0
40253	4·5	40263	—
40236	4·5	40343	6·2
40255	4·7	40262	4·8
40256	5·3	40266	4·3
40257	4·4	40254	4·5
40258	4·9	40268	4·5
40259	4 6	40269	4·5
40260	4 4	40333	5·4
AVERAGES	4.7	AVERAGES	4·9

Apparently the total yield of wool (not scoured) was not influenced by sulphur feeding. In view of the negative results of the detailed analysis of the wool of the sheep receiving a daily dose of sulphur in the earlier investigation (du Toit *et al* 1934) only the fleece weights were taken in this experiment. It was conclusively shown in the earlier work (van Wyk *et al* 1935) that sulphur feeding is without effect on the quality and quantity of the wool produced.

DISCUSSION.

On comparing the weight increases of the two groups of sheep it will be noticed that the mean increases are reasonably equal there being only a difference of ·11 kg. in favour of the sulphur fed group which is altogether insignificant. When allowance is made for the difference in food intake it is found that this adjustment does not make an appreciable reduction in the variance and that the adjusted difference of 2·2 kg. in favour of the control group remains quite insignificant.

In short, it may be said that the conclusion is justified that a daily dose of 5 grms. of flowers of sulphur is without effect on the bodyweight, food consumption and wool production of sheep. It seems necessary, however, to review briefly the work on sulphur feeding to sheep that has been published from this Institute in order to state the final conclusions clearly.

Steyn's investigations (1931, 1932, 1934 and 1935) were undertaken, as stated by him, to determine the quantity of sulphur that could be administered to young and full-grown sheep with safety over prolonged periods. Obviously, the body weights of the sheep could be registered with advantage and this was done accordingly. Furthermore, the sheep had to be shorn in the course of the experiment, and hence, the weights of the wool produced were recorded. It is abundantly clear from Steyn's publication (1934) that he fully realizes the unsuitability ot his material to determine the nutritional value of sulphur when, naturally, increase in body weight, food consumption, effect on production, etc., are important considerations and if this information had been required his experiments would have been planned differently so as to to ensure the registration of these observations for the collection of suitable data for justifiable and definite conclusions. At best, therefore, it can be said that the results of Steyn's original experiment (1931, 1932) seemed to suggest that sulphur when administered to mature sheep at the rate of 5 grms. once, twice or three times weekly, over prolonged periods stimulates increase in body weight and wool yield. In a subsequent experiment (Steyn 1935) 5 grms. of sulphur were administered daily to immature sheep without detrimental effect on body weight and on the wool production of the animals. As a matter of fact the differences in body weight and in the weights of the wool produced are significantly in favour of the sulphur fed animals. All the sheep were fed *ad lib.* as one group, which excludes a consideration of more efficient food utilization or increased food consumption of the sulphur fed when compared with the controls. Again, therefore, it can be said that this investigation suggested that sulphur feeding to sheep affects their bodyweights and wool production beneficially. What is, however, quite definite about this work is that sulphur administered in the doses stated did not affect the animals detrimentally.

Working upon the indications of Steyn's original experiments (1931, 1932) du Toit *et al* (1935) carried out an investigation with sheep to determine the nutritional value of sulphur. Five grms. of of sulphur were dosed to each animal in a group of sheep daily while another group was kept as controls. The experiment was planned to satisfy the requirements of a feeding trial. The body weights of the sheep were registered periodically, a productive ration was fed to the individual sheep in separate feeding boxes and the food consumption of the individual animals was recorded. The animals were kept on the basal ration for a twelve months pre-experimental period after which the sulphur was administered to the one group for a further twelve months. Both groups were of course still kept on the basal ration for the second period of twelve months. Wool was collected from all the animals in both groups, analysed and reported on by van Wyk, Botha and Bekker (1935). In view of the entire absence of any significant difference in body weight, food consumption and wool production of the sulphur fed group when compared with the control group the conclusion appears to be justified that a daily dose of 5 grms. sulphur did not effect the sheep significantly, when they were given a production ration.

The above experiment with sheep on a limited food intake obviously excludes a consideration of increased food consumption which might be an effect of sulphur feeding and hence the investigation reported on in the present paper under conditions of *ad libitum* feeding was undertaken. Again the experiment was planned, primarily with the object of determining the nutritional value of sulphur and fulfills the demands of a feeding trial.

As in the previous work no beneficial effect of sulphur feeding could be determined, nor was the food intake of the sulphur fed group significantly affected.

The main result of the Onderstepoort experiments on the effect of administering sulphur to sheep, could therefore be summarized as follows: Five grms. of sulphur can be given *per os* to sheep over prolonged periods without detrimental effects. The body weights, food consumption and wool production are apparently not significantly affected by the sulphur administered to the sheep.

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Sulphur Metabolism.

II. The Distribution of Sulphur in the Tissues of Rats fed Rations with and without the addition of Elementary Sulphur.

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THE injection and oral administration of elementary sulphur as a prophylactic in various diseases has been practised for many years and the popularity of this drug in medicine seems to increase of late rather than decrease. Yet, curiously enough, little was known about the extent to which this element is absorbed and stored in the animal body. However, a few investigators have studied the storage of some of its metabolic products (sulphide and sulphate) but, unfortunately, their results do not give a true picture of the metabolism of elementary sulphur as such. Nevertheless, their results have some bearing on those to be presented in this article and are, therefore, reviewed.

It was found at the Iowa Experimental Station (1924) that sulphur, when fed to ewes in daily doses of 0·5 oz., was retained in the animal body to a considerable extent, though there was evidence that some had been oxidised to sulphates and excreted in the urine. Support seems to be lent to this point of view by the author's work (1935) on the absorption and excretion of sulphur in rats and sheep. Denis and Reed (1927b) analysed the blood of dogs that had been fed a standard ration to which had been added powdered sulphur to the extent of 0·5 gm. per kilo. per day and found in some cases an increase in the non-protein sulphur compounds and in others a marked decrease. Tasaka and Nakazawa (1931) observed that the change in blood picture after subcutaneous injection of colloidal sulphur is similar to that after subcutaneous injection of insulin, only less marked. According to the work of Kubo (1931) the feeding of sulphur and injection of insulin decidedly increased both the total and neutral sulphur contents of most organs, especially those of the suprarenal capsules where the sulphate content was also raised. Meyer-Bisch and Techner (1931) found no changes in the hydrolyzable and total sulphur contents of the liver in rabbits that received sulphur parenterally.

Denis and Leche (1925b) in a series of experiments in which hypertonic solutions of sodium sulphate were administered to dogs by intravenous injections found by analysis of the blood, muscles, and viscera that there was little absorption of the sulphate iron by the tissues, although even two hours after injection the sulphate content of the blood might still be ten times its initial value. Arnoldi, Liss and Rosam (1932) fed mineral water containing neutral sulphates to one and alkaline sulphates to another group of rats and found that the average values for total sulphur in the livers of the animals were higher for both the groups given sulphate waters than that of the control animals.

Neither Lehmann (1892) nor Meyer (1898) could find any hydrogen sulphide in the blood of dogs that had inhaled this gas over a long period of time. Denis and Reed (1927a) analysed the blood of dogs that had received sodium sulphide by intravenous injection, or by injection into the intestine and observed that there was sometimes a rise in the inorganic sulphate but no increase in the neutral sulphur fraction. These increased values were noted particularly in the case of animals in whom the kidneys had been tied off.

Taken as a whole the literature given above seems to show that sulphur is stored to a certain extent in the animal body and it was, therefore, the object of this investigation to study more fully the extent to which and the form and tissues in which sulphur is stored in the organism, which information, it was thought, might subsequently throw some light upon the cause of the toxic effect of the oral administration of flowers of sulphur as observed by Steyn (1931), Lewis and Lewis (1927) and Lawson, Redfield and Boyce (1934).

EXPERIMENTAL.

White rats were used in these experiments. The young rats used in the first experiment were the same animals that were employed in another investigation in which the growth of rats fed a modified Osborne-Mendel diet with and without added sulphur was studied. The composition of the rations and the experimental conditions are given in the third paper (Kellermann, 1936), of this series and will, therefore, not be described in detail again. For the sake of clarity, however, it can be mentioned that three groups of young rats were fed a modified Osborne and Mendel low protein ration with and without the addition of 0·8 per cent. of elementary sulphur. Groups II and III received the sulphur supplemented ration. In addition the animals received distilled water except those of group III that were given fresh orange juice *ad libitum* instead of water. On the last day of the experiment referred to above the rats were killed and the tissues removed for analysis. Two groups of 64 and 68 adult rats of about nine to ten months of age were used in the second experiment. They were raised on the stock ration used in this laboratory (Kellermann, 1934) and the only difference in the treatment of these groups was the substitution of three parts by weight of flowers of sulphur for the same amount of yellow maize meal in the ration of one of the groups for the last twenty-one days.

The rats were put under ether anaesthesia and then bled to death after severing one or both carotid arteries. The blood was collected in a beaker that contained lithium citrate as anticoagulant. After the pooled blood was strained through muslin in order to remove any hairs, it was allowed to cool down to room temperature. The brain, femoral muscles and thoracic and abdominal viscera were removed, and great care was taken not to contaminate them with any hairs. The tissues to be analysed were then pooled and finely ground up.

Only the total sulphur determinations were made on the tissues of the rats (young) in the first experiment. They were made on the dry materials, after the tissues had been dried in an electric oven. The tissues of the rats (adult) in the second experiment were analysed for total sulphur, total sulphate and volatile sulphide. All the determinations were made on the fresh tissues, but for the sake of better comparison the results are expressed in milligrams sulphur per 100 gm. dry material. The moisture determinations were carried out in 35 c.c. weighing bottles kept for about twelve to fifteen hours in an electric oven which was run at 98° C. The total sulphur determinations were made according to the method of Feigl and Schorr (1923). When the determination was made on the fresh material the ground up tissue (2-3 gm.) was thoroughly mixed with the Na_2CO_3-$KMnO_4$ mixture and then dried over a steam bath before the rest of the method was proceeded with. The total sulphate sulphur determinations were made according to a modified method of Denis and Leche (1925a). Whenever enough material was available, 20 gm., instead of 10 gm., of fresh material were autoclaved, as described by Denis and Leche, with 200 c.c. of 25 per cent. Hcl (i.e. 1 vol. of concentrated Hcl made up to 4 vols. with distilled water) in a 400 c.c. Jena beaker. The contents of the beaker were then transferred quantitatively to a 500 c.c. volumetric flask by warm distilled water, cooled and the flask filled to the mark. The liquid, after thorough mixing was filtered through No. 588 of Carl Schleicher and Schull's filter paper. The sulphate in an aliquot (250 c.c.) of the filtrate was then precipitated by the addition of $Bacl_2$ solution and determined further according to the gravimetric method as $BaSo_4$. The volatile sulphide was also determined gravimetrically as $BaSO_4$ as described by Heffter and Hausmann (1904). The results obtained with the young rats are given in Tables I, II and III.

TABLE I.

Distribution of Total Sulphur in Tissues of Young Rats fed Modified Osborne-Mendel Ration for Sixty Days.

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Sulphur in Dry Matter.
	Per Cent.	Mgm. Per Cent.
Brain	77·9	521·1
Lung	77·5	593·6
Liver	71·6	582·2
Spleen	75·1	647·1
Heart	77·3	856·5
Kidney	74·7	726·4
Muscle	72·3	680·1
Blood	81·6	878·0

TABLE II.

Distribution of Sulphur in Tissues of Young Rats Receiving Elementary Sulphur in Food. (Modified Osborne-Mendel Ration Containing 0·8 per cent. Flowers of Sulphur for Last Sixty Days.

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Total Sulphur in Dry Matter.
	Per Cent.	Mgm. Per Cent.
Brain	77·5	532·6
Lung	76·9	**763·8**
Liver	69·6	563·9
Spleen	73·9	**796·9**
Heart	76·5	925·5
Kidney	74·3	797·9
Muscle	73·3	673·9
Blood	80·7	887·0

TABLE III.

Distribution of Sulphur in Tissues of Young Rats Receiving Elementary Sulphur in Food. (Modified Osborne-Mendel Ration Containing 0·8 per cent. Flowers of Sulphur Plus Orange juice ad libitum *for last Sixty Days.)*

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Total Sulphur in Dry Matter.
	Per Cent.	Mgm. Per Cent.
Brain	77·5	525·9
Lung	73·6	**644·2**
Liver	72·3	551·6
Spleen	63·5	**747·3**
Heart	72·7	832·4
Kidney	70·8	748·7
Muscle	71·0	665·6
Blood	81·3	804·0

It will be seen that in the first group without the addition of elementary sulphur the blood and heart contained the largest quantity of sulphur. Next in order came the kidney, then the muscle and spleen, and lastly the lung, liver and brain. Comparison of the data in Tables II and III with those in Table I shows that sulphur feeding increased especially the total sulphur content of the lung and spleen, and also to a certain extent that of the heart and kidney in group II. The lungs and spleen of the sulphur fed animals in group II contained respectively 170·2 and 149·8 mgm. and in group III 50·6 and 100·2 mgm. more sulphur per 100 gm. dry material than the same tissues taken from animals fed the basal ration alone. The hearts and kidneys of the animals in group II only contained 69·0 and 71·5 mgm. per cent. more sulphur than the respective organs of the control group. On the other hand the livers and muscle of the sulphur fed animals contained less sulphur than the same tissues from the control animals. However, these reduced values are so small that they are of no significance. Furthermore, it should be mentioned that on the whole, less sulphur was stored in the tissues of group III than in those of group II. It may be that the extra basic elements introduced with the orange juice caused a faster and more complete excretion of the oxidised sulphur in the urine in view of the fact that Moraczewoki and Sliwinski (1934) have found that the elimination of both thiocyanates and sulphates is rapid on an alkaline diet but slow and somewhat prolonged on an acid one. Unfortunately, the data available are too few and the differences in the amounts of sulphur stored in the various tissues of rats fed sulphur rations with and without orange juice are not marked enough to warrant any definite conclusions and further work is necessary in order to elucidate this point.

It should also be pointed out that the accuracy of the results presented in Tables I to III must be accepted with some reservation in view of the fact that the tissues were first dried before being analysed and it is possible that during the process of drying some of the sulphur escaped as volatile sulphide. In order to test out this point two groups each of five young rats were fed the modified Osborne and Mendel low protein ration, with and without the addition of 0·8 per cent. elementary sulphur, over a period of 60 days after which their tissues were analysed for volatile sulphide. The latter was tested for qualitatively on the various fresh tissues (pooled for each group) by steam distillation as described by Osborne (1928). Notwithstanding the fact that the animals were in a very poor condition, no volatile sulphide could be detected in any of the tissues by this method except in the liver where only a trace was present. Bearing the observations of Osborne in mind, it would seem, therefore, that fresh rat tissues and beef in poor condition, unlike the flesh of sheep and guinea-pigs in a similar condition, do not emit sulphide on boiling. The liver of the sulphur-fed animals emitted about three times as much sulphide as that of the control group, whereas only traces of sulphide were found in the kidney and muscle of the sulphur group. Furthermore, twenty-four hours after killing, the muscle of the control group did not yet emit any sulphide whereas that of the sulphur group showed about twice as much sulphide by that time as the fresh muscle from the same group. With the probable exception of the liver of the sulphur group, it would seem, therefore, that he sulphide lost through drying could not have been of such magnitude that it would have seriously affected the validity of the conclusions drawn.

As a matter of fact the results presented in Tables IV and V show that negligible amounts of H_2S were present in the fresh tissues of adult rats that received a stock diet with and without the addition of sulphur.

TABLE IV.

Distribution of Various Forms of Sulphur in Tissues of Adult Rats Receiving Stock Ration.

(Sixty-four animals, about nine to ten months of age, were used.)

Tissue.	Moisture.	Total S.	Sulphate S.	Hydrogen Sulphide S.	"Undetermined". S.
	Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.
Brain............	77·1	751·5	77·87	1·03	672·60
Lung............	75·2	**803·9**	30·45	trace	773·45
Liver............	72·0	895·6	11·79	0·68	883·13
Spleen............	75·5	**1110·0**	**11·21**	trace	1098·79
Heart............	77·2	**1364·0**	9·63	,,	1354·37
Kidney............	75·8	1062·0	**30·12**	,,	1031·88
Muscle............	70·8	933·3	4·24	,,	929·06
Blood............	79·2	876·8	10·64	,,	866·16

TABLE V.

Distribution of Various Forms of Sulphur in Tissues of Adult Rats Receiving High Intake of Elementary Sulphur. (Stock Ration containing 3 per cent. of Flowers of Sulphur for last Twenty-one Days.

(Sixty-eight animals, about nine to ten months of age, were used.)

Tissue.	Moisture.	Total S.	Sulphate S.	Hydrogen Sulphide S.	"Undetermined" S.
	Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.
Brain............	77·4	764·3	81·41	0·99	681·90
Lung............	76·2	**861·8**	27·39	0·25	834·16
Liver............	72·3	925·5	16·76	0·50	908·24
Spleen............	75·7	**1171·0**	**19·77**	trace	1151·23
Heart............	77·1	**1445·0**	15·02	,,	1429·98
Kidney............	75·4	1088·0	**50·75**	1·36	1035·89
Muscle............	72·4	958·1	7·47	0·30	950·33
Blood............	78·6	883·9	18·91	0·36	864·63

A further study of these tables shows that the heart contained the largest quantity of total sulphur. Next in order came the spleen and kidney, then the muscle, liver, blood and lung with brain the lowest. Exactly the same order also holds true for the sulphur group. Comparison of the data in Table V with those in Table IV shows that sulphur feeding increased the total sulphur content of the heart, spleen and lung. These organs of the sulphur fed animals contained, respectively, 81·0, 61·0 and 57·9 mgm. more sulphur per 100 gm. dry matter than the same organs taken from animals raised on the stock ration alone. This substantiates the data obtained with young rats (Tables I and II) fed the Osborne-Mendel ration with and without added sulphur. However, it should be pointed out that the total sulphur contents of the tissues of the adult rats fed the stock ration alone are all, except for th blood, perceivably higher than those of the same tissues of young rats fed the basal Osborne-Mendel ration. The grand average of the tissues of the eight organs for the adult and young rats were 974·6 and 685·6 mgm. per 100 gm. dry material, respectively. It should be borne in mind that the young rats were fed a low protein, cystine deficient diet that was also very low in sulphur content whereas the adult rats were raised on a normal stock ration, and one of the contributing factors for the low total sulphur content for the tissues of the young rats probably was that the tissues of the latter animals were consequently very low in non-protein sulphur.

The data of Kambayashi (1929) seem to support this theory His figures for the total sulphur, expressed as a percentage of the fresh material, are slightly lower than those of the author, calculated on the same basis, for adult rats. Unfortunately, he only determined the total sulphur in the femoral muscles and liver of male rats weighing 180 to 250 gm. For these tissues he found 242 and 222 mgm. per cent., respectively, whereas the author's figures are 188·4 and 165·3 mgm. for the respective tissues of the young and 272·5 and 250·8 mgm. for those of adult rats. From these figures it is obvious that his data, obtained by means of fusing the dry material with a mixture of KNO_3 and HNO_3 etc., are likewise appreciably higher than the results obtained by the author with the young rats, the cause of which, as mentioned before, should most probably be looked for in the low sulphur intake by these animals.

With regard to the total sulphate content of the tissues in the control group the brain contained by far the largest amount of this salt. Next in order came the lung and kidney, with approximately equal amounts, then the liver, spleen and blood also with approximately equal amounts whereas the heart and muscle contained the least. The high figure for kidney can, as mentioned by Denis and Leche (1925b) probably be explained on the basis of unavoidable inclusion of traces of urine, while it is possible that the high values for the brain and lung were due to the decomposition of some unstable sulphur containing lipoid. Comparison of the sulphate content of the kidney, spleen and blood. These tissues of the sulphur fed animals contained, respectively, 20·63, 8·55 and 8·27 mgm. more sulphate sulphur per 100 gm. dry material than the same organs from animals fed the stock ration alone.

With regard to the distribution of hydrogen sulphide, it is clear that the feeding of sulphur had no effect upon the concentration of this fraction in the tissues. This is to be expected in view of the fact that Haggard (1921) has shown that blood plasma possesses the property of oxidizing hydrogen sulphide rapidly by the withdrawal of oxygen from the blood corpuscles. Although he did not determine the products of oxidation, results obtained by the author (1936) seem to show that they are sulphates. This finding, together with the observation of Denis and Leche (1925b) that sulphates, injected into the blood stream, are only slowly excreted, explain why the blood and most tissues of sulphur-fed rats contain more of this constituent than the tissues from control animals.

The " undetermined " sulphur values of the tissues of both the sulphur and control groups ran parallel, as will be expected, with the total sulphur contents, and comparison of the former values in Tables IV and V shows once more that feeding of elementary sulphur increased especially the sulphur content of the lung, spleen and heart.

SUMMARY.

(1) The distribution of sulphur was determined in the tissues of three groups of young rats. One was fed a modified Osborne-Mendel low protein basal diet, one the basal diet supplemented with 0·8 per cent. of elementary sulphur, and the remaining one the same supplemented ration plus orange juice *ad libitum*. Sulphur feeding increased especially the total sulphur content of the lung and spleen. Next in order came the heart and kidney. The total sulphur content of the lung of the sulphur group was 170·2 and of the spleen 149·8 mgm. more per 100 gm. dry material than the content of the respective tissues taken from the control group. The average increase in the amount of sulphur in the heart and kidney was about half of the average increase in the lung and spleen. On the whole less sulphur was stored in the tissues of the sulphur group that received orange juice in addition, but the differences are too small to warrant any definite conclusions.

(2) The distribution of various forms of sulphur in the tissues of two groups of adult rats,, one fed the stock ration, the other the stock ration supplemented with 3 per cent. elementary sulphur, was also determined. Sulphur feeding likewise increased the total sulphur content of the heart, spleen and lung but not so much of the kidney. The three former organs contained, respectively, 81·0, 61·0 and 57·9 mgm. more sulphur per 100 gm. dry material than the same organs taken from animals raised on the stock ration alone.

(3) Sulphur feeding had little effect upon the sulphate content of rat tissues, thus substantiating the observation of Denis and Leche (1925b). The only tissues in which the sulphate content was slightly increased were the kidney, spleen and blood. The respective tissues of the sulphur fed animals contained 20·63, 8·55 and 8·27 mgm. more sulphate sulphur per 100 gm. dry material than the same tissues taken from the control animals.

(4) There was found to be no difference in the concentration of volatile sulphide in the tissues of animals fed rations with and without the addition of flowers of sulphur.

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Sulphur Metabolism.

III. The Effect of Flowers of Sulphur on the Growth of Young Rats fed an otherwise Well-balanced Ration.

By J. H. KELLERMANN, Section of Biochemistry and Nutrition, Onderstepoort.

According to the work of several investigators the oral administration of flowers of sulphur offers interesting possibilities of success in checking intestinal putrefaction in chicks in confinement (Phillips, Carr and Kennard, 1921) and worm infestation in sheep (Steyn, 1932). Montgomery (1932) and Burman (1932) also found that the ingestion of an adequate amount of flowers of sulphur served as a good prophylactic in malaria.

Unfortunately the administration of sulphur cannot be left in inexperienced hands and the promiscuous feeding of sulphur should be warned against, in view of the fact that the dosing of high levels of sulphur was found to be definitely toxic. Steyn (1931), for instance, observed that the dosing of 45 gm. of flowers of sulphur "per week per sheep over a period of thirty-five days given in daily doses of 15 gm. cause symptoms of poisoning and high mortality." Lawson, Redfield and Boyd (1934), who experimented with rabbits and guinea pigs, found that by giving doses of from 0·5 to 2·0 gm. of either colloidal or flowers of sulphur per kgm. of body weight directly into the stomach through a small stomach tube all the animals died within twenty-four hours. The stomach walls were necrotic, the kidneys intensely congested and the livers slightly so. The spleens showed little change as did the hearts and lungs. Lewis and Lewis (1927) observed that sulphur when added to the milk powder-starch diet of Sherman and Merrill (1925) was definitely toxic and caused the death within periods of from 3 to 21 days of fourteen out of twenty-two animals whose diet contained it to the amount of 1 per cent. With a few exceptions, the livers of the rats which died showed a marked peripheral zonal necrosis.

However, the latter authors also observed that the feeding of 1 per cent. of sulphur with the Osborne-Mendel low protein diet was less toxic than when added to the same extent to the Sherman-Merrill diet. This observation seems to show that the toxicity of sulphur can be controlled to a certain extent by the composition of the food given. If that is true, it will be of great practical importance in regions where sulphur is administered as a prophylactic to disease in man and animals.

As will be pointed out later on in this article both the Sherman-Merrill and Osborne-Mendel diets are deficient in certain food essentials whereas the mixed diets of man and animals, under normal conditions, usually contain enough of the various essential constituents. It was thought, therefore, of interest to study the effect of elementary sulphur on the growth of young animals given diets, which were as complete as possible, from a nutritional standpoint.

EXPERIMENTAL.

Albino rats of about three to four weeks old and of the London strain of the Wistar Institute stock were used in these experiments. In the first experiment (preliminary) three groups of five rats each were used. Each group of animals was housed in a big cage with a raised screen bottom. The animals were fed *ad libitum* and the total food consumption for each group recorded. The basal ration used in this experiment was a modification of the Osborne-Mendel low protein diet given in Table I. One group received the basal ration and the other two the basal ration supplemented with 0·8 per cent. of flowers of sulphur. The sulpur was added at the expense of the dextrinized starch. The animals were weighed once a week throughout the experimental period and on the last day of the experiment. Fresh distilled water was at all times available to the animals except to those of one of the sulphur groups which received fresh orange juice *ad libitum* instead of water. The orange juice was substituted for water because it was thought that its basic elements might play a rôle in the detoxication of hydrogen sulphide. As a matter of fact the author (1936) found that H_2S is oxidized (detoxicated) better in an alkaline than in an acid or neutral medium. If this is also true for the oxidation of H_2S in living organisms, the observations of Saywell and Lane (1933) and Schuck (1934) that the hydrogen ion concentration of the urine of men and women subjects can be decreased and the alkaline reserve of their bodies increased by taking 1,000 c.c. of orange juice daily might still prove itself of value in the detoxication of sulphur. The results are given in Table II.

TABLE I.

Composition of Basal Rations in Percentage by Weight.

	Modified Osborne-Mendel Diet.	Stock Diet.
Merk's casein	10	—
Crude casein	—	5
Modified Steenbock salts 40*	4·5	—
Dextrinized starch	54·5	—
Powdered sucrose	5	-
Merk's prepared lard	19	—
Cod liver-oil	3	—
Agar	2	—
Brewer's yeast	2	5
Yellow maize meal	—	68
Linseed oil meal	—	6
Milk powder (Nestle's, S. Africa)		4
Lucerne meal	—	3
Butter fat	—	5
Beef liver (dried at 70° C.)	—	2
Bone ash	-	1
Calcium carbonate	—	0·5
Sodium chloride		0·5

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* The $MgSO_4 \cdot 7H_2O$ in Steenbock and Nelson Salts 40 (1923) was replaced by the same amount of magnesium carbonate.

TABLE II.

Summary of Growth and Food Consumption.

Rat. No.	Sex.	Diet.	Initial Weight.	Final Weight.	Gain or Loss.	Total Food.	Gain per 100 gm. Food.
			Gm.	Gm.	Gm.	Gm.	Gm.
11	Female	Basal	43	56	+13	—	—
12	,,	,,	52	84	+32	—	—
13	,,	,,	45	72	+72	—	—
14	,,	,,	50	69	+19	-	--
15	,,	,,	53	73	+20	—	—
		MEAN	48·6	70·8	+22·2	262	8·5
16	Male	Basal + 0·8 per cent. S.	50	47	− 3	--	-
17	,,	—	44	54	+10	—	—
18	,,	—	41	43	+ 2	—	—
19	,,	—	46	57	+11	—	--
20	,,	—	36	57	+21	—	--
		MEAN	43·4	51·6	+ 8·2	226	3·6
21	Female	Basal + 0·8 per cent. S. plus orange juice *ad lib.*	48	78	+30	—	—
22	,,	—	40	54	+14	—	—
23	,,	—	40	35	− 5	—	-
24	,,	—	47	54	+ 7	—	—
25	,,	—	44	44	± 0	—	—
		MEAN	43·8	53	+ 9·2	222*	4·1

* The food value of orange juice was not taken into consideration.

In view of the fact that the groups were too small and, because the food consumption of each animal was not measured separately, no statistical analysis can be made of the results. However, the results seem to show that the incorporation of 0·8 per cent. of sulphur in the basal ration retarded the rate of growth, and that the administration of orange juice had, under the experimental conditions, no beneficial effect on the rate of growth of the second sulphur group. Over a period of 60 days the average gain in weight, the total food consumed and the gain per 100 gm. food were 22·2, 8·2 and 9·2; 262, 226 and 222, and 8·5, 3·6 and 4·1 gm. for the basal, sulphur and sulphur plus orange juice groups respectively.

In a second experiment the effect of different concentrations of flowers of sulphur was studied on the growth of young rats fed a basal ration, which in the light of present knowledge, is complete in all respects for this species of animal. The diet was a slight modification of the stock ration (Kellermann, 1934) used in this laboratory. Its composition is given in Table I. The sulphur rations had the same composition as the basal except that sulphur was used in the place of like amounts of yellow maize meal in the diets. The paired-feeding method of Mitchell and Beadles (1930) was employed, and all pair-mates were matched for litter and sex. There were six pairs to each group. The initial and final weights of the rats were the average of weighings taken on three consecutive days. During the rest of the experimental period they were weighed once a week. Fresh distilled water was given in addition to the ration. The animals were fed according to the method of Mitchell (1933). The results are given in Table III.

It will be seen that on the whole the control rats gained highly significantly more in weight than those receiving the sulphur supplement. The number of weeks, however, during which the control rats gained more in weight than their mates is reasonably near 50 per cent. and therefore, indicates no such difference as reflected by the gains in weight for the entire period. Furthermore, the various treatments show no significant difference amongst themselves.

It is also of interest to note that on the whole the rats in this experiment, including those that received rations containing added sulphur, made as good gains as those obtained by Greenman and Duhring (1923) for the albino rat under normal conditions. As a matter of fact even the 11 male and 13 female rats fed the basal ration in which was incorporated elementary sulphur in such high concentrations as 2·0, 2·5, 3·0 and 3·5 per cent. reached at approximately 82 days of age average weights of 206·5 gm. and 148·2 gm., respectively, which were 60·8 gm. and 11·2 gm. above the respective normal weights given by Greenman and Duhring.

DISCUSSION.

Lewis and Lewis (1927) advanced the theory that the lessened toxicity of sulphur on the Osborne-Mendel diet as compared with the Sherman-Merrill ration might be explained by its higher fat and protein contents. However, it should be pointed out that, as a

TABLE III.

Growth Records of Rats on Basal Ration with and without Different Percentages of Elementary Sulphur.

(All periods, 8 weeks, and all weights in grams).

Percentages of Elementary Sulphur incorporated.	Data.	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
		Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.
0·1	Initial weight	54	52	54	55	60	62	61	61	65	64	62	61
	Final weight	166	162	153	153	158	158	149	161	292	283	235	223
	Total gain	112	110	99	98	98	96	88	100	227	219	173	162
	Total food	559	558	522	520·5	522	522	513	513	816	816	668	668
	Comparison of weekly gains	4	4	3	5	5	3	3	5	5	3	4	4
0·25	Initial weight	55	56	59	59	73	69	80	79	65	68	57	57
	Final weight	162	167	268	282	287	279	298	303	173	172	167	163
	Total gain	107	111	209	223	214	210	218	224	108	104	110	106
	Total food	534	533·5	757	757	769	768·5	795	795	570	570	540	537·5
	Comparison of weekly gains	4	4	3	5	5	3	3·5	4·5	4·5	3·5	5	3
0·5	Initial weight	61	62	61	61	74	76	71	71	74	77	75	77
	Final weight	169	171	252	245	265	261	287	278	243	251	218	221
	Total gain	108	109	191	184	191	185	216	207	169	174	143	144
	Total food	539	539	673	673	708	708	768	768	698	696	654	654
	Comparison of weekly gains	6	2	5·5	2·5	5·5	2·5	5	3	3	5	4·5	3·5
0·75	Initial weight	76	75	62	63	88	90	66	65	58	58	60	61
	Final weight	240	250	143	140	285	288	160	154	171	170	164	167
	Total gain	164	175	81	77	197	198	94	89	113	112	104	106
	Total food	726	726	483	483	775	775	550	550	596	596	562	562
	Comparison of weekly gains	2	6	4	4	4	4	5	3	4	4	3	5
1·0	Initial weight	53	54	66	69	80	78	73	73	68	70	53	56
	Final weight	150	148	147	158	246	242	240	227	248	233	247	234
	Total gain	97	94	81	89	166	164	167	154	180	163	194	178
	Total food	518	518	529	529	685	685	651	651	742	742	669	669
	Comparison of weekly gains	4	4	2	6	3	5	5	3	7	1	5	3
1·25	Initial weight	56	57	67	69	66	65	50	50	47	51	63	66
	Final weight	159	153	179	195	153	150	145	136	132	130	228	228
	Total gain	103	96	112	126	87	85	95	86	85	79	165	162
	Total food	523	521	593	593	492	492	229	230	468·5	469	657	657
	Comparison of weekly gains	4	4	3	5	4·5	3·5	5	3	5	3	4	4

TABLE III—(*continued*).

Percentages of Elementary Sulphur incorporated.	Data.	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
		Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.
1·5	Initial weight............	61	61	50	53	49	51	40	40	52	55	54	54
	Final weight............	162	154	210	195	151	157	142	136	152	166	228	225
	Total gain.............	101	93	160	142	102	106	102	96	100	111	174	171
	Total food.............	530	530	586	586	516·5	517	481	481	537	537	638	637·5
	Comparison of weekly gains	4·5	3·5	6	2	3·5	4·5	4·5	3·5	3	5	5	3
2·0	Initial weight............	56	57	62	63	59	59	48	49	73	72	48	50
	Final weight............	164	166	271	252	147	152	128	131	222	216	219	203
	Total gain.............	108	109	209	189	88	93	80	82	149	144	171	153
	Total food.............	607	607	750	750	483	483	468	471	675	675	594	594
	Comparison of weekly gains	3·5	4·5	6·0	2·0	3·5	4·5	5·5	2·5	4·5	3·5	5·5	2·5
2·5	Initial weight............	62	62	64	62	65	65	61	60	80	80	69	70
	Final weight............	207	192	183	184	145	131	151	139	234	215	148	139
	Total gain.............	145	130	119	122	80	66	90	79	154	135	79	69
	Total food.............	563	563	564	564	462	462	522	523	635	635	481	481
	Comparison of weekly gains	6·0	2·0	4·0	4·0	6·0	2·0	6·0	2·0	5·5	2·5	5·5	2·5
3·0	Initial weight............	69	68	66	67	58	58	60	61	72	73	63	63
	Final weight............	150	147	158	151	179	163	160	145	258	246	155	156
	Total gain.............	81	79	92	84	121	105	100	84	186	173	92	93
	Total food.............	537	537	553	553	593	593	506	506	762	762	540	540
	Comparison of weekly gains	4·0	4·0	4·5	3·5	6·0	2·0	6·0	2·0	5·5	2·5	4·0	4·0
3·5	Initial weight............	78	79	72	69	71	70	79	79	78	78	61	62
	Final weight............	141	152	185	172	159	158	245	224	240	221	134	144
	Total gain.............	63	73	113	103	88	88	166	145	162	143	73	82
	Total food.............	453	453	501	501	522	522	688	688	655	655	480	482
	Comparison of weekly gains	3·0	5·0	4·0	4·0	5·0	3·0	6·5	1·5	4·5	3·5	2·0	6·0

whole, the Osborne-Mendel diet is better balanced than the Sherman-Merrill one. Not only are its protein and salts about twice as high as those of the latter diet but it is also rich in both vitamins A and D. The Osborne-Mendel diet, on the other hand, is decidedly lower in the vitamin B complex than the Sherman-Merrill diet, consequently, in this particular case vitamin B can be ruled out as an agent in the detoxification of sulphur. Furthermore, it is very improbable that the fat in the Osborne-Mendel diet played any appreciable rôle in the detoxification of the sulphur in view of the fact that it could not be concluded from previous results (Kellermann, 1935) that lard had an inhibitory effect on the absorption of sulphur. If, as suggested by Lewis and Lewis, the fat formed a coating around the particles of sulphur and thus made more difficult the intimate contact of the sulphur with the intestinal mucosa or the action of bacteria, one would have expected a lower concentration of hydrogen sulphide in the digestive tract and consequently a lower absorption of sulphur.

There remains one more point of difference in the composition of the Osborne-Mendel and Sherman-Merrill diets that deserves to be pointed out, namely, their contents in easily available " food sulphur ". In view of the fact that the composition of the Osborne-Mendel salt mixture (1913) conforms closely to that of milk salts as such, it is evident that the sulphur content of the Osborne-Mendel basal diet must have been at least four times as high as that of the Sherman-Merrill one. Furthermore, it will be remembered that the inference was drawn in a previous publication (Kellermann, 1935) that the presence of easily available " food sulphur " in a ration might exert a depressing effect upon the toxicity of elementary sulphur, and it is possible that the greater content of " food sulphur " of the Osborne-Mendel diet accounted partly for the greater antitoxic effect of sulphur poisoning.

From a perusal of the results given in Tables II and III, it is evident that, on the basis of every 100 gm. of food consumed, the rats on the modified Osborne-Mendel diet gained over 50 per cent. more in weight than those on the same diet supplemented with 0·8 per cent. sulphur; whereas in no case were such great differences observed in the growth of pair-mates fed the modified stock ration with and without sulphur. As a matter of fact, in only a few instances did the control rats gain as much as 12 to 13 per cent. over their " sulphur " mates on the same amount of food, and in 28·8 per cent. of the total number of pairs, the sulphur-fed animals gained even more than their control mates. Furthermore, it should be borne in mind that the sulphur-containing rations had a lower energy value than the basal diet, and the rats in the sulphur groups were, therefore, put at a disadvantage to those in the basal groups in so far as growth was concerned.

In the 0·75 and 1·0 per cent. sulphur groups, in contrast to those that received 0·8 per cent. sulphur with the Osborne-Mendel diet, there was practically no difference in the average gains made by the control and " sulphur " groups. It is evident, therefore, that the stock ration has much more antitoxic effect to sulphur poisoning than the Osborne-Mendel diet. The latter diet, as modified in this experiment, meets, as far as is known, all the essential requirements for growth except that it is low in protein and cystine contents. It would seem that its low cystine content could be ruled out as a cause of the high toxicity of sulphur, in view of the fact that Lewis and Lewis (1927) found that the toxicity of sulphur, when incorporated in the cystine deficient diet of Osborne and Mendel, was not affected by the addition of amounts of cystine adequate to produce good growth. However, this diet is also low in easily available " food sulphur " for the reason that the Osborne-Mendel salt mixture was replaced by the same amount of Steenbock and Nelson salts 40 (1923), modified slightly so as to contain no sulphur. Furthermore, it was found by the author (1935) that the absorption of sulphur in a ration containing 10 per cent. of casein and 2 per cent. of brewer's yeast, as the only sources of food sulphur, was rather low with a subsequently high absorption of elementary sulphur and it might be, therefore, that the high toxicity of sulphur when fed with the Osborne-Mendel diet as compared with its low deleterious effect in the stock ration, was partly due to the low protein and " food sulphur " contents of the former diet.

SUMMARY.

1. Experiments are described in which were studied the effect of flowers of sulphur on the growth of young white rats maintained on two types of basal diets, one a modification of the low protein diet of Osborne and Mendel and the other a ration complete in all respects from a nutritional standpoint for this species of animal.

2. Sulphur, when added to the amount of 0·8 per cent. to the low protein diet which was also low in cystine and " food sulphur ", caused a marked retardation of the rate of growth but no deaths during an experimental period of 60 days.

3. The toxicity of sulphur when added to a well balanced ration in concentrations varying from 0·1 to 3·5 per cent. was, comparatively speaking, rather low. A comparison of the rate of growth of young rats by the paired-feeding method of Mitchell and Beadles showed that the number of weeks during which the control rats gained more in weight than their litter mates on the sulphur rations, is reasonable near 50 per cent., which indicates that the different treatments show no significant difference amongst themselves.

4. The solution of the problem as to why certain rations show greater antitoxic effect to sulphur poisoning than others should probably be looked for in the difference in their protein and " food sulphur " contents. The results to date seem to show that rations, complete in all respects for growth with optimum protein and easily available " food sulphur " contents, possess greater antitoxic effect to sulphur poisoning than rations that are low in these constituents.

5. Large amounts of flowers of sulphur can be given to animals (rats) without marked deleterious effects on their rate of growth when optimum amounts for growth of a well-balanced ration (stock) is given at the same time.

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Sulphur Metabolism.

IV. The Oxidation and Reduction of Elementary Sulphur by Animal Tissues *in vitro*.

By J. H. KELLERMANN, Section of Biochemistry and Nutrition, Onderstepoort.

In an article on the therapeutic value of sulphur, Bridges (1934) stated that " sulphur is one of the oldest of drugs and still occupies an important place in our *materia medica* . . . Sulphur has played an important part in human medicine, and its use in veterinary medicine is increasing every day ".

Unfortunately, little is as yet known how it functions therapeutically. Power (1930; 1932) studied in some detail the effects of colloidal sulphur injections both in man and in rabbits, and found that one of its most characteristic effects appears to be a leukocytosis of polymorphonuclear cells in the blood stream. Furthermore, he found that these cells exhibit, under *in vitro* conditions, a considerable degree of phagocytic activity. However, Meyer-Bisch and Basch (1921) observed that marked differences exist between the effects of the oral and parenteral administration of sulphur, as determined by the composition of the urine. The fact that the latter form of administering sulphur together with its external application as an ointment in the treatment of eczema, mange, ringworm, etc., is used so extensively in medicine, probably lead to the study of the factors that influence the reduction of elementary sulphur by animal tissues *in vitro*. The reduction of sulphur is most probably the first metabolic change that sulphur undergoes in its sojourn in the organism. Evidently the better the factors that influence this initial step are understood, the easier it will be to study the subsequent transformation of sulphur.

Heffter (1904) and Heffter and Hausmann (1904) found that in the absence of bacteria and enzymes egg white, blood and many other animal tissues, including the intestinal mucosa, could form hydrogen sulphide from elementary sulphur. However, in a strongly acid solution the production of H_2S was completely inhibited. Sluiter (1930) confirmed the results of Heffter and believes the conclusion to be justified that H_2S production in living tissue after addition of sulphur is not due to enzyme activity. She seems to be of opinion that the sulphide production can be explained undoubtedly by the purely chemical process: $2\ G.SH + S = G.S.S.G. + H_2S$, in view of the fact that tissues, glutathione and a dehydrogenase solution, as three different sources of SH used by her, all yielded H_2S after addition of sulphur. Likewise, Di Capua (1934) observed that sulphur is reduced to H_2S at about 30° in the presence of cystine, and De Rey-Pailhade (1929) found that H_2S is evolved when certain biological materials are mixed with sulphur at 40°, even if the nitroprusside reaction is not given.

The subsequent detoxication of the H_2S seems to be by oxidation to sulphate, and the blood is doubtless the main tissue of the animal body in which oxidation takes place. The results of Denis and Reed (1927a) give support to this theory. Furthermore, Haggard (1921) found that the rate of oxidation of H_2S in the blood is such that in a comparatively short period many times the lethal amount of sodium sulphide may be administered intravenously to animals without any apparent effect. The blood is reduced by the H_2S as a result of the withdrawal of oxygen from the corpuscles for the oxidation of the sulphide. He found that the products of oxidation, though undetermined, combine in part with the sodium of the plasma. Another tissue of importance in the oxidation of sulphur appears to be the suprarenal glands. Loeper, Garcin and Lesure (1926) believe that the suprarenals possess a double function, namely, thiopexic and thio-oxidizing in view of the fact that blood from the suprarenal vein contains much less sulphur than that from the suprarenal artery, whereas blood from the suprarenal vein contains a larger proportion of oxidized sulphur than does that from the suprarenal artery. Moreover, they observed that the suprarenals are richer in sulphur than most other organs, which corroborates the results of Aufrecht and Driesing (1910) who consider the adrenal glands as the regulators of the sulphur metabolism of the animal body just as, for instance, the thyroid gland regulates iodine metabolism.

It is evident from the results of Denis and Reed (1927b), substantiated later by the work of the author (1935) that he absorption of elementary sulphur from the alimentary tract results mainly in its excretion in the urine as sulphates. According to the observations of Meyer-Bisch and Basch (1921) a large fraction of the sulphur injected peritoneally or intramuscularly is also excreted in the sulphate form. Unfortunately, not much could be learned from these *in vivo* studies as to the extent to which certain factors might influence the metabolism of sulphur, and it was, therefore, the object of this paper to investigate some of the factors that might influence the reduction and especially the oxidation of elementary sulphur by animal tissues *in vitro.*

EXPERIMENTAL.

In all of the experiments on the reduction (and oxidation) of elementary sulphur by animal tissues *in vitro*, a control was always run on the same tissue without the addition of sulphur, and under the same experimental conditions, in so far as time and temperature were concerned. This precautionary measure was essential in view of the fact that Osborne (1928) has shown that the flesh of several species of animals, when starved or in an emaciated condition, emitted H_2S immediately after killing whereas the flesh of well-fed animals of the same species did not evolve H_2S until some hours, preferably twenty-four, after killing. Furthermore, in order to exclude the possibility of any bacterial action, the studies were carried out in an antiseptic solution. With the exception of one experiment where a weak antiseptic of 5 gm. boracic acid per litre of Ringer solution was used, all the experiments were carried out with the use of a very strong antiseptic of 0·83 gm. thymol per litre of Ringer solution (Lutman 1929).

The production of H_2S by animal tissues in T.R. solution* was studied under seven different conditions. The first solution constituted a negative control to which no sulphur was added. The next two served as sulphur controls but the tissue in one of them was first boiled for 30 minutes in order to destroy all enzyme action. To the four remaining solutions were added sulphur plus varying amounts of acid and alkali to give a solution approximately N/4·3 with respect to HCl, N/1·76 with respect to NaOH and N/10 with respect to Na_2CO_3 and $NaHCO_3$ respectively. When phenolphthalein was used as indicator and the solutions containing beef liver were titrated hot to end-point with HCl or NaOH as necessary, it was found that the first three solutions had on the average an acid equivalence of 2·23 c.c. N.HCl, the fourth of 38·14 c.c. N.HCl whereas the last two had an average base equivalence of 10·73 c.c. of N.NaOH. Almost identical figures were obtained with beef muscle. The base equivalence of the sodium carbonate and bicarbonate solutions were determined by adding an excess of acid to the solution which, after being boiled to drive off the carbon dioxide, was then titrated back with alkali. The difference between the amount of N.HCl added and that of N.NaOH used in the back titration constitutes the base equivalence of the solution. The base equivalence of the fifth solution, which was made strongly alkaline by the addition of NaOH, could not be determined in view of the fact that no sharp end-point could be obtained.

The determination of the reduction of elementary sulphur by animal tissues was carried out as follows: 50 gm. of tissue or 50 c.c. of blood plus 150 c.c. T.R. solution together with the necessary chemicals, as the case may be, and to be described shortly, were introduced into a 700 c.c. Pyrex boiling flask and stoppered with a tight fitting rubber stopper which was provided with in and out leading glass tubes. The end of the tube through which air was later to be drawn in, was such that its end dipped under the surface of the solution in the flask. To the ends of the in and out leading tubes were attached tight fitting rubber tubing, the free ends of which were closed tightly with screw pinch-cocks. The flasks were then kept in an incubator, which was run at 37°, for twenty-four hours with occasional shaking after which the free volatile sulphide was determined by the method as described by Heffter and Hausmann (1904). However, an error may possibly be introduced by the reduction of elementary sulphur by the various solutions as such, that is, without the presence of tissue, in view of the fact that several investigators [Geitner, (1864); Boehm, (1883); and Cross and Higgin, (1883), as quoted by Heffter and Hausmann (1904)] have found that small amounts of H_2S were given off when sulphur was boiled with water. Likewise, Heffter (1904) found that sulphide was formed when sulphur was heated at 40° with either a sodium hydroxide or carbonate solution.

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* T.R. solution is an abbreviation for thymol-Ringer solution.

In order, therefore, to see whether elementary sulphur was reduced by the antiseptic solutions as such, these were put through the same routine as described above and determinations made of the amounts of H_2S emitted. Negative results were found for most of the solutions except for the carbonate and bicarbonate ones from which an average of only a small fraction of a milligram of H_2S-S was obtained. As a result of these minimal values in comparison with the large amounts of sulphide formed by liver and muscle, they were simply disregarded in the presentation of the results obtained with tissues. The results are given in Table I.

It will be seen that only traces of H_2S were emitted by the blood in the various solutions. The most probable reason for this phenomenon is that the H_2S was completely oxidized by the oxygen present in the blood corpuscles. However, quite different results were given by the liver and muscle. Here the addition of sulphur alone caused the formation of an average increase of 98·7 mgm. H_2S-S per cent. over and above the negative control. Boiling caused a great average decrease of 59·86 mgm. H_2S-S per cent. compared with the fresh tissue yet still 38·87 mgm. per cent. higher than the value of the negative control. The decrease in H_2S formation after boiling of the tissue can probably be explained on the grounds that the SH group was decomposed by heating as was shown by Sluiter (1930) and the $2\ G.SH + S = G.S.S.G. + H_2S$ reaction could, therefore, no longer take place. In a strongly acid or alkaline solution the amount of H_2S emitted was markedly decreased. This substantiates the observations of Heffter and Hausmann (1904) and Sluiter (1930). In the case of the acid solution the liver and muscle gave an average value of only 9·32 mgm. H_2S-S per cent. whereas practically none was recovered from the strongly alkaline solution. However, under the experimental conditions, it cannot be concluded that no reduction took place in the strongly alkaline medium, in view of the fact that the H_2S formed might have been completely bound by the excess of alkali. Later results seem to give indirect testimony to this effect. In the presence of a weak alkaline solution the production of H_2S was markedly enhanced. The average increases over the sulphur control were 364·2 and 148·7 mgm. H_2S-S per cent. for the sodium carbonate and bicarbonate solutions, respectively.

In order to see to what extent the alkali as such was responsible for the increase in H_2S formed, a control was run, simultaneoulsy, for each of the various solutions. The results given in Table II show that only the $NaHCO_3$ solution, as such, caused an increase in the amount of H_2S formed. In this case the average amount (for liver and muscle) of H_2S emitted was only 36·3 mgm. H_2S-S more than that of the negative control. On the other hand when sulphur was added the amounts of H_2S evolved by the sodium hydroxide, carbonate and bicarbonate solutions were nil, 462·7 and 202·0 mg. H_2S-S per cent. more than those of their respective

TABLE I.

The Reduction of Elementary Sulphur by Various Beef Tissues in T.R. Solution.

	Tissue.	Moisture.							
		Per Cent.			Boiled for 30 mins.				
Treatment of Tissues:									—
Per cent. S added to fresh tissues........	--	—	--	3	3	3	3	3	3
Normality of solution with respect to HCl..	-	—	—	—	--	N/4·3	—	—	—
Normality of solution with respect to NaOH	--	—	—	—	-	—	N/1·76	—	—
Normality of solution with respect to Na_2CO_2	—	—	—	--	—	—	—	N/10	—
Normality of solution with respect to $NaHCO_2$	—	—	—	—	—	—	—	—	N/10
Mgm. H_2S. S per 100 gm. dry tissues.....	Blood	81·56	trace	trace	trace	trace	trace	trace	trace
Mgm. H_2S. S per 100 gm. dry tissues.....	Aerated* Blood	79·39	,,	,,	,,	,,	,,	,,	,,
Mgm. H_2S. S per 100 gm. dry tissues.....	Liver	71·12	4·67	96·56	76·79	4·74	,,	812·3	100·7
Mgm. H_2S. S per 100 gm. dry tissues.....	Muscle	76·33	15·34	120·90	20·95	10·90	,,	133·5	414·1

* After the T.R. solution and the necessary chemicals were added to the blood in the boiling flask, the latter was immediately connected up in the apparatus for the determination of hydrogen sulphide and H_2S-free air drawn through for three hours. The H_2S emitted was caught up in an ammoniacal H_2O_2 solution. The boiling flask was partly immersed in a water bath kept at a constant temperature of 40° C.

TABLE II.

The Effect of Alkali on the Reduction of Elementary Sulphur by Animal Tissues in T.R. Solution.

	Tissue.	Moisture			Increase in H_2S. S.			Increase in H_2S S.			Increase in H_2S S.			Increase in H_2S S.
		Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.
Per cent. S added to fresh tissue	—	—	—	3	—	—	3	—	—	3	---	—	3	—
Normality of solution with repect to NaOH	—	—	—	—	—	N/1·76	N/1·76	—	—	—	—	—	—	—
Normality of solution with respect to Na_2CO_3	—	—	—	—	—	—	—	—	N/10	N/10	—	—	—	—
Normality of solution with respect to $NaHCO_3$......	—	—	—	—	—	—	—	—	—	—	—	N/10	N/10	—
Mgm. H_2S-S per 100 gm. dry material......	Beef Liver	71·12	4·7	96·6	91·9	trace	trace	0	5·4	812·3	806·9	17·9	82·7	64·8
Mgm. H_2S–S. per 100 gm. dry material......	Beef muscle	76·33	15 3	120·9	105·6	trace	trace	0	15·0	133·5	118·5	74·8	414·1	339·3

controls. It is evident, therefore, that the large amount of H_2S rendered by the carbonate and bicarbonate solutions, containing elementary sulphur, owes its origin chiefly to the sulphur present and only to a very small degree to the reducing effect of the salt (bicarbonate) upon the tissue itself. Nevertheless, the addition of the carbonate and bicarbonate salts increased considerably the reduction of elementary sulphur by animal tissues. This is clear from the fact that the reduction of elementary sulphur, as such, by tissues in the carbonate and bicarbonate solutions exceeded on the average the reduction of sulphur by the same tissues in an ordinary T.R. solution by 364·0 and 103·3mgm. H_2S-S per cent., respectively.

In order to see to what extent various tissues can also oxidize elementary sulphur, the amount of sulphate present in the different solutions, after twenty-four hours of incubation, was also determined. Two different types of solution were employed, one with weak (0·5 per cent. boracic acid) and the other with very strong (0·083 per cent. thymol) antiseptic properties. The experimental conditions were exactly the same as already described under the determination of the reduction of elementary sulphur, except that the solutions with the tissues, chemicals, etc., were kept in 400 c.c. Gena glass beakers and, during incubation, occasionally stirred with glass rods. The negative control solutions, that is, those to which no elementary sulphur was added, were always incubated in a separate incubator in order to prevent the absorption of any volatile sulphide. After incubation the solutions were acidified with HCl, autoclaved and the sulphate determined gravimetrically, as $BaSO_4$, by the method as described by the author (1936). However, caution should be exercised not to ascribe oxidizing properties to tissues that actually belong to the antiseptic solutions as such. For that reason blank determinations were made of the amount of sulphates formed by the various acid and alkaline solutions, to which sulphur was added. In all of the solutions, except for the sodium hydroxide one, the amounts of sulphates formed were either nil or so small that they might be disregarded. However, appreciable amounts of sulphate were formed in the hydroxide solution. For three different determinations values ranging from 0·0368 gm. to 0·0376 gm. with an average of 0·0372 gm. $BaSO_4$ were obtained. This average value had of course to be deducted from the amount of $BaSO_4$ precipitated in each of the tissue-hydroxide solutions.*

The data presented in Table III show that he boracic acid and T.R. solutions gave parallel results. Furthermore, comparison of the sulphate values in Table III with those of the H_2S in Table I reveals a close parallelism between these data. This suggests that the oxidation of elementary sulphur is preceded by its reduction, and that the same factors which influence its reduction will, therefore, also show up in its oxidation, in view of the fact that the greater the concentration of H_2S formed the more of it will be available for oxidation and *vice versa*. For the sake of simplicity only the average values of the various columns will be discussed.

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* The values for sulphate-sulphur given under the tissue-hydroxide columns in the tables represent those after the necessary corrections had been made.

TABLE III.

The Oxidation of Elementary Sulphur to Sulphate by Animal Tissues in Weak and very Strong Antiseptics.

In 0·5 per cent. Boric Acid Solution

	Tissue.	Animal.	Moisture.							
			Per Cent.							
Treatment of Tissue:						Boiled for 30 mins.				
Per cent. S added on basis of fresh tissue......	—	—	—	—	3	3	3	3	3	3
Normality of solution with respect to HCl....	—	—	—	—	—	—	N/4·3	—	—	—
Normality of solution with respect to NaOH....	—	—	—	—	—	—	—	N/1·76	—	—
Normality of solution with respect to Na_2CO_3...	—	—	—	—	—	—	—	—	N/10	—
Normality of solution with respect to $NaHCO_3$..	—	—	—	—	—	—	—	—	—	N/10
Mgm. SO_4–S per 100 gm. dry tissue..........	Blood	Bovine	80·98	8·51	20·81	7·09	6·62	36·87	28·38	25·54
,, ,, ,, ...	Dried (100°C.) liver	Sheep	69·58	3·61	3·61	3·61	3·61	58·23	5·42	6·78
In Thymol-Ringer Solution.										
Mgm. SO_4–S per 100 gm. dry tissue....... ...	Liver	Beef	70·33	15·74	18·99	17·13	12·50	240·3	95·85	37·51
,, ,, ,, ...	Steamed (3 hrs.) liver	,,	66·37	8·98	8·98	—	trace	247·0	31·03	32·67
,, ,, ,, ...	Dried (70° C.) liver	,,	7·45	7·72	8·31	9·49	6·53	150·2	12·08	10·97
,, ,, ,, ...	Muscle	,,	75·61	1·69	13·39	trace	1·13	212·4	98·02	33·81
,, ,, ,, ...	Blood	,,	81·71	14·71	47·07	32·85	12·75	371·6	194·60	115·20
,, ,, ,, ...	Liver	Rat	70·87	2·83	3·77	2·83	1·89	302·8	43·40	22·64
,, ,, ,, ...	Muscle	,,	72·23	trace	0·49	1·48	trace	137·6	53·43	19·79
,, ,, ,, ...	Small intestine	,,	77·80	3·71	3·71	—	4·95	—	14·86	—
,, ,, ,, ...	Blood	,,	79·11	1·28	60·67	—	—	—	—	—
Average mgm. SO_4–S per 100 gm. dry tissue....	—	—	—	6·25	17·25	9·31	5·0	195·22	57·70	33·88

The addition of sulphur to the tissues resulted in part in its being oxidized to sulphate. This is evident from the fact that the tissue-sulphur solutions (sulphur controls) yielded 11·0 mgm. more sulphate-sulphur per cent. than those (negative controls) to which no sulphur was added. Previous boiling of the tissues, however, reduced the oxidizing capacity of the tissue-solutions because those containing tissues so treated only yielded 3·06 mgm. more sulphate-sulphur per cent. than the negative controls. In a strongly acid medium this oxidizing capacity is completely lost whereas the addition of alkalies, on the other hand, greatly enhanced the oxidation of elementary sulphur by animal tissues. This is evident from the fact that from the sodium hydroxide, carbonate and bicarbonate solutions an average of 177·97, 40·45 and 16·63 mgm. more sulphate-sulphur per cent. were recovered than from the sulphur controls.

In order to see to what extent the alkalies, as such, were responsible for the marked increase in sulphates formed, a control, without the addition of sulphur, was run for each solution. The results are given in Table IV.

It is clear, that the addition of any of the alkalies, increased the sulphate content of tissues when determined after an incubation period of twenty-four hours. On the average for bovine blood and liver the sodium hydroxide, carbonate and bicarbonate solutions yielded, respectively, 46·9, 6·0 and 7·1 mgm. more sulphate-sulphur per cent. than the control solutions to which no alkali had been added. However, when sulphur was added in addition to the alkalies, the average increases in sulphate values, as a result of the sulphur as such, that is, the increases over the alkali controls to which no sulphur was added, were 243·8, 124·0 and 54.0 mgm. sulphate-sulphur per cent. for the sodium hydroxide, carbonate and bicarbonate solutions, respectively. These values still exceed the average net sulphate increase due to sulphur, without the addition of alkali, by 226·0, 106·2 and 36·2 mgm. sulphate-sulphur per cent. for the respective alkaline solutions from which it is evident that, although alkali in itself increases the sulphate content of tissues, it nevertheless augments the oxidation of elementary sulphur by animal tissues to a marked extent.

Exactly how the alkali facilitates the oxidation of elementary sulphur by tissues is not quite clear. It is evident that its action is not secondary to any vital entity in the tissue, in view of the fact that some of the studies were carried out on tissues previously steamed or dried (see Table III) and always under strictly antiseptic conditions. The results suggest, however, that the chief effects of the alkali in the oxidation of sulphur are to increase the reduction of sulphur by the tissue and the subsequent concentration of sulphide in the solution; furthermore, it is well known that both sodium and hydrogen sulphide are rather unstable in solution and gradually take on atmospheric oxygen to form sulphate. It might be, therefore, that the sole effect of the alkali was to increase the reduction and concentration of sulphide in the solution where it was oxidized either quickly by the oxygen or corpuscles, in the case of blood, or slowly by atmospheric oxygen, in the case of " blood free " tissues. If this theory is correct, it sould also follow that the oxidation of a

Table IV.

The Effect of Alkali on the Oxidation of Elementary Sulphur by Animal Tissues in T.R. Solution.

	Tissue.	Moisture. Per Cent.			Increase in SO_4S. Mgm. Per Cent.			Increase in SO_4S. Mgm. Per Cent.			Increase in SO_4S. Mgm. Per Cent.			Increase in SO_4S. Mgm. Per Cent.
Per cent. S added to fresh tissue	—	—	—	3	—	—	3	—	—	3	—	—	3	—
Normality of solution with respect to NaOH	—	—	—	—	—	N/1·76	N/1·76	—	—	—	—	—	—	—
Normality of solution with respect to Na_2CO_3	—	—	—	—	—	—	—	—	N/10	N/10	—	—	—	—
Normality of solution with respect to $NaHCO_3$	—	—	—	—	—	—	—	—	—	—	—	N/10	N/10	—
Mgm. SO_4–S per 100 gm. dry material	Bovine blood	81·71	14·7	47·1	32·4	80·8	371·6	290·8	23·0	194·6	171·6	19·1	115·2	96·1
Mgm. SO_4–S per 100 gm. dry material	Bovine liver	70·33	15·7	19·0	3·3	43·5	240·3	196·8	19·4	95·8	76·4	25·5	37·5	12·0

" constant " supply of H_2S by an alkaline-tissue solution should be the same no matter whether the tissue was fresh, boiled or dried. This was found to be actually the case, and demonstrated as follows: a " constant " stream of H_2S was passed for exactly fifteen minutes through one of each two samples of fresh, boiled and dried tissue in 150 c.c. of N/10 (with respect to Na_2CO_3) T.R. solution. The H_2S-treated solutions were then incubated separately from the untreated (control) ones and after an incubation period of twenty-four hours, with occasional stirring, the sulphate in each was determined as described previously. The difference between the sulphate contents of the H_2S-treated and untreated solutions constitutes the amount of sulphate formed from the oxidation of hydrogen sulphide. The results are given in Table V.

TABLE V.

The Oxidation of Hydrogen Sulphide By Animal Tissues in T.R. Solution, N/10 *with respect to Sodium Carbonate.*

State of Tissue.	Tissue.	Fresh.	Boiled. (30 mins.)	Dried. (100° C.)
Mgm. SO_4-S formed from oxidation of H_2S	Beef liver....	1·63	2·06	1·79
	Beef muscle	1·61	1·39	1·29

The similarity of the values obtained with the differently treated tissues is striking. For beef liver they varied from 1·63 to 2·06 mgm. with an average of 1·83 mgm., and in the case of beef muscle from 1·29 to 1·61 mgm. with an average of 1·43 mgm. What is of interest is the fact that the amount of sulphate-sulphur recovered from three blanks of alkaline (N/10) Thymol-Ringer solutions through which H_2S was also passed, etc.,varied from 4·205 to 4·479 mgm. with an average of 4·30 mgm., which is 163·8 per cent. more than the grand average of the liver and muscle values. A probable explanation as to why the addition of tissues reduced the capacity of the alkaline solution to oxidize H_2S, might be that the formation of sodium proteinates reduced the dissociation of the salt with a subsequent reduction in the power of the solution to bind and concentrate the volatile sulphide.

Because of the large amounts of carbonates and buffer salts present in blood where injected (intravenously) sulphur is reduced (Heffter 1904) and then readily oxidized (Haggard 1921), it was thought of interest to study also the influence of these salts, in the absence of tissues, on the oxidation of H_2S. This experiment was carried out as follows: A " constant " stream of H_2S was passed for exactly fifteen minutes through one of each of two 200 c.c. samples of the following solutions: thymol-Ringer solutions with and without the addition (to give N/10 solutions) of sodium hydroxide, carbonate and bicarbonate. Similarly, H_2S was passed through one of each of two samples of the following buffer mixtures, KH_2PO_4—NaOH (Clark 1922); Na_2HPO_4—KH_2PO_4, and sodium borate and

N/10 HCl (Sörensen 1909). The pH values of these mixtures are 7·4, 7·347 and 7·621, respectively, all of which approximate closely that of blood. The H_2S-treated and control solutions were incubated separately after which the amounts of sulphate were determined.

TABLE VI.

The Oxidation of Hydrogen Sulphide to Sulphate in 200 *c.c. of Various Alkaline and Buffer Solutions.*

In T.R. Solutions.

Normality with respect to NaOH	—		N/10		—		—	
Normality with respect to Na_2CO_3	—		—		N/10		—	
Normality with respect to $NaHCO_3$	—		—		—		N/10	
Saturated with..................	—	H_2S	—	H_2S	—	H_2S	—	H_2S
Mgm. SO_4–S in sample..........	0·08	0·71	0·05	1·39	0·05	0·81	0·069	0·74

In Buffer Solutions.

	KH_2PO_4—NaOH Mixture.		Na_2HPO_4—KH_2PO_4 Mixture.		Na borate and N/10 Hcl Mixture.	
H ion concentration (pH)..	7·4		7·347		7·621	
Saturated with...........	—	H_2S	—	H_2S	—	H_2S
Mgm. SO_4–S in sample....	0·05	1·55	0·15	1·72	0·19	1·25

The data presented in Table VI show that the H_2S-treated thymol-Ringer solution and those made N/10 with respect to sodium hydroxide, carbonate and bicarbonate contained, respectively, 8·87, 27·8, 16·2 and 10·72 times as much sulphate as the control solutions without the addition of H_2S. It is evident, therefore, that the oxidation of H_2S was markedly increased by the alkaline salts added. This was most probably accomplished through the formation of the non-volatile sodium sulphide* which, like the H_2S, in T.R. solution, was slowly oxidized to sulphate when exposed to atmospheric oxygen. For similar reasons the oxidation of H_2S in the buffer solutions was also greatly increased. The H_2S-treated buffer solutions contained, in the order given previously, 31·0, 11·45, and 6·58 times as much sulphate as the H_2S-free solutions.

If alkaline salts can exert such far reaching effects on the reduction and subsequent oxidation of elementary sulphur, by tissues *in vitro,* they should also have the same influence on orally administered sulphur, in view of the fact that sulphur is readily reduced by the bacteria and mucosa of the digestive tract in whose contents oxygen is normally always present. As a matter of fact, it was observed by the author (1935) that the intestinal contents of rats, fed elementary sulphur in addition to a protein-free ration, were much richer in sulphates than the contents of animals fed the same ration without the addition of sulphur. It was suggested at that time that the increase in sulphate " might have been due to a more copious flow of intestinal secretions induced by hydrogen sulphide irritation, or to a greater concentration of sulphates in the secretion of the sulphur-fed rats, or to both ". In the light of knowledge gained later in an *in vitro* study, to be presented directly, it seems as though this theory is only partly true because the greater part of the increase in sulphate content most probably had its origin in the digestive tract through the oxidation of H_2S to sulphate.

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* That solutions of $NaHCO_3$ are capable of binding and concentrating H_2S as Na_2S was, according to Pohl (1886-87), demonstrated experimentally by Diakonow.

The *in vitro* study was carried out as follows: a group of twelve adult rats were fed *ad lib.* distilled water and a protein and salt free ration consisting of dextrinized starch 62, sucrose 20, lard 10, cod liver oil 2, and agar 6 parts by weight for eight days. They were then killed and the contents of the stomach, small intestine and caecum removed. The pooled contents of each section were then divided, after samples for moisture determination had been taken, into three parts as nearly as possible of the same weight. To one part was added elementary sulphur and to another an alkaline salt mixture in addition to the sulphur. The salt mixture was a modification of Steenbock and Nelson salts 40 (1923) in which the $MgSO_4 7H_2O$ was replaced by an equivalent amount of $MgCO_3$. After thymol-Ringer solution was added to each part, they were incubated at 37° for twenty-four hours and their sulphate contents determined. The results are given in Table VII.

TABLE VII.

The Oxidation of Elementary Sulphur to Sulphate by the Intestinal Contents of Rats, in T.R. Solution.

Contents.	Stomach.			Small Intestinal.			Caecal.		
Moisture per cent.	49·86			84·89			87·16		
Weight of fresh sample (gm.)..	27	27	27	25	24	21	7·5	8	8
No. of c.c. T.R. solution added	65	65	65	20	20	20	20	20	20
Per cent. S added on basis of dry material......	—	3·69	3·69	—	4·13	4·73	—	14·56	14·56
Per cent. salts 40* added on basis of dry material	—	—	5·17	—	—	11·04	—	—	33·98
Mgm. SO_4–S per 100 gm. dry material......	63·13	62·52	75·30	252·3	264·2	287·7	458·0	466·9	547·0

*** The inorganic salt mixture added was a modification of Steenbock and Nelson salts 40 (1923) in which the $MgSO_4 \cdot 7H_2O$ was replaced by an equivalent amount of $MgCO_3$.**

It will be seen that the addition of sulphur alone to the stomach material had no effect on its sulphate content which was probably due to the acid nature of the stomach contents. On the other hand when 5·2 per cent. of salts were added in addition, the sulphate content increased 12·17 mgm. sulphate-sulphur per cent., as compared with that of the control solution. The addition of sulphur and sulphur plus salts to the contents of the small intestine increased their sulphate values by 11·9 and 35·4 mgm., and when added to the caecal contents by 8·9 and 89·0 mgm. sulphate-sulphur per cent. respectively, when compared with the sulphate values of their control solutions. Furthermore, the parallelism between the amounts of salts added and the subsequent increases in sulphates formed, as compared with those of the sulphur controls, is striking. The amounts of salts added to the contents of the stomach,small intestine and caecum were 5·17, 11·04 and 33·98 per cent., respectively, and the subsequent increases in sulphate were 12·78, 23·5 and 80·1 mgm. sulphate-sulphur. When the lowest amount of salts added is taken as unity, and the two other values are expressed as multiples thereof, their relation to each other is as 1 : 2·1 : 6·6; similarly, the relation of the subsequent sulphate increases is as 1 : 1·8 : 6·3 to each other which shows how closely the oxidation of elementary sulphur is dependant upon the alkalinity of the medium. The importance, therefore, of an excess of base-forming elements in the diet is evident, especially in cases where a large amount of H_2S is formed in the digestive tract, in view of the fact that these elements help to oxidize and detoxicate part of the sulphide before it reaches the liver. On the other hand, it should be mentioned that alkalies also increase the reduction of elementary sulphur which might largely offset their beneficial effects when sulphur is incorporated in the ration.

Discussion.

The experiments described in this paper show that elementary sulphur is first reduced to sulphide and subsequently oxidized to sulphate by animal tissues *in vitro*, and that both processes are inhibited by acids but enhanced by alkalies and alkaline salts. In view of the fact that all protein (SH) containing tissues can reduce elementary sulphur, it is clear that the only other condition necessary for its subsequent oxidation is that the tissue be bathed in an alkaline (or alkaline buffer) solution which is also rich in oxygen. If the oxidizing and reducing reactions studied in the laboratory are analogous to those taking place in living organisms, it is evident that the essential conditions given above are excellently fulfilled in the blood stream. Nevertheless, by measuring the H_2S content of the expired air, it was found by Forst (1928) that H_2S was formed in the blood in amounts approximating the fatal dose after the intravenous injection of relatively large amounts of colloidal sulphur, and it is probably for that reason that at the present time the injection of sulphur either subcutaneously or intramuscularly is more and more resorted to.

When sulphur is injected in this way, it is probably first reduced to sulphide, as in the case of blood, then partly oxidized and finally, as the results of Meyer-Bisch and Basch (1921) seem to show, excreted largely as sulphates in the urine. The question now arises as to how the oxidation of the sulphide to sulphate is accomplished in the organism. Part of it, no doubt, passes into the blood stream and is oxidized there whereas some of it is most probably oxidized by the tissue into which it is injected without the aid of the blood stream, in view of the fact " that hydrogen peroxide is a product of cell activity and that phosphates are present in all cells" (Gortner 1929) thereby containing in itself the essential agents for the oxidation of sulphide. However, the tissues into which sulphur is usually injected are much poorer in oxygen and buffer salts than blood, and it is there that one might be able to enhance the oxidation and reduction of elementary sulphur by the simultaneous injection of alkaline salts. If the enhancing effect of alkaline salts on the reduction of sulphur by animal tissues *in vitro* also holds true for its reduction in living organisms, the injection of a colloidal suspension of sulphur in an alkaline solution might find special application in certain cases of acute poisoning. It is revealed in the literature (Kellermann 1935) that colloidal sulphur forms a good antidote for corrosive sublimate and cyanide poisoning in view of the formation of the less poisonous sulphide and thiocyanate salts, respectively. It is evident, therefore, that if the liberation of the labile sulphur (sulphide) could be increased and speeded up, it should be possible to render a dose of poison, several times the lethal amount, quite harmless. If this theory is correct, it would help to explain why Forst (1928) found the administration of such a large amount (three to four times lethal dose) of HCN harmless when followed up within ten to fifteen minutes by the injection of a colloidal suspension of sulphur in a sodium bicarbonate solution.

SUMMARY AND CONCLUSIONS.

1. Data are presented on the oxidation and reduction of elementary sulphur by animal tissues *in vitro*, and the enhancing or otherwise effects of certain chemicals on these reactions are also shown.

2. All protein tissues seem to be capable of reducing sulphur to H_2S. Acid (HCl) inhibits this reaction whereas alkalies and alkaline salts enhance it.

3. Similarly, acid inhibits and alkalies and alkaline buffers increase the oxidation of reduced sulphur to sulphate. An alkaline medium, rich in oxygen, is therefore essential for the oxidation and detoxication of H_2S. This is readily accomplished in blood where these conditions are excellently fulfilled. Nevertheless, all tissues in the animal organism should be able to oxidize sulphides, in view of the fact that oxygen (e.g. H_2O_2) and phosphates (buffers) are present in all tissues.

4. The chief, if not the sole, effect of alkali on the oxidation of sulphur seems to be to bind and concentrate the reduced sulphur in the solution where it is then oxidized to sulphate by the oxygen present in the corpuscles, in the case of blood, or by atmospheric oxygen, in the case of other tissues. Furthermore, the oxidation of sulphur by tissues under laboratory conditions is not influenced by enzyme activity, in view of the fact that no difference was found in the oxidation of H_2S by fresh, boiled or dried tissue suspensions in an alkaline (Na_2CO_3) thymol-Ringer solution. As a matter of fact the oxidation of H_2S also took place with relative ease in alkaline (and buffer) solutions without the presence of tissues.

5. Similarly, the reduction of sulphur by animal tissues is not due to enzyme activity because the reaction took place in tissues that were boiled previous to their incubation with sulphur in a very strong antiseptic solution.

6. A close parallelism was found to exist, under laboratory conditions, between the increases in the oxidation of sulphur and (as a result of) the different percentages (on the dry basis) of modified Steenbock salts 40 added to the intestinal contents of the rats fed an ash and protein-free ration. This suggests that a diet with an excess of base-forming elements may be of importance in the oxidation and detoxication of sulphides in cases where large amounts of H_2S are formed in the digestive tract.

7. The possibility of beneficial effects arising from injecting (subcutaneously or intramuscularly) a colloidal suspension of sulphur in an alkaline solution as an antidote in certain cases of acute poisoning is discussed.

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Section VI.

Sex Physiology.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies in Sex Physiology, No. 14.

The Situation of the Developing Foetus in the Uterus of the Live Merino Sheep.

By H. H. CURSON and J. QUINLAN, Section of Surgery and Obstetrics including Sex Physiology, Onderstepoort.

In a previous study (Curson and Quinlan, 1934), the opinion was expressed that the most suitable time to gain knowledge concerning the situation of the developing foetus would be during life. Such an opportunity presented itself when sheep D.O.B. 37782 and D.O.B. 37931 were made available for X-ray examination.

According to Arey (1931) bone begins to appear in man after the seventh week, so the first attempt to X-ray the developing foetus was left to the 59th day (Sheep 37782), but no foetal skeleton was observed. Actually centres of ossification in the Merino foetus may be seen in the metacarpal and metatarsal bones as early as the 45th day.

While the lateral recumbent position would have suited best for photography, it was considered that the most reliable picture would be obtained by photographing the uterus transversely while the ewe was in the standing position. In this way uterine displacement was obviated.

The ewe, during the taking of the photographs, was kept on a ration low in roughage (a little teff hay), so as to reduce the transverse diameter of the rumen. In addition ¾ lb. crushed oats, ½ lb. crushed maize, and a little green barley *per diem* was given. No food or water was allowed during the 24 hours prior to photographing.

The ewe was strapped loosely in the standing posture to a rotary Potter-Buchy diaphragm. The exposure was as follows:—K.V. 90; M.A. 200; time 0·2 seconds; distance 30 inches and supra-screens were used. Longer exposures than 0·2 seconds were unsatisfactory owing to the blurring of the negative by respiratory movements and secondary rays.

Penetration of the contents of the rumen is not easy at such low kilovoltages as will not completely penetrate the developing bones.

The situation of the foetuses at the various intervals was as follows:—

Ewe 37782 (*served* 21*st August*, 1933.)

Fig. 1 (73 days). *Presentation* posterior; *position of foetus* lumbo-iliac.

Fig. 2 (87 days). *Presentation* anterior; *position of foetus* dorso-ilio-pubic.

Fig. 3 (107 days). *Presentation* anterior; *position of foetus* dorso-sacral with lateral cephalic rotation.

Fig. 4 (129 days). *Presentation* posterior; *position of foetus* lumbo-pubic.

Fig. 5 (134 days). *Presentation* anterior; *position of foetus* dorso-iliac.

On the 142nd day (10th January, 1934), a ram lamb (D.O.B. 38853) was born.

Ewe 37931 (*served* 5*th September*, 1933.)

Fig. 6 (92 days). *Presentation* anterior; *position of foetus* dorso-iliac.

Fig. 7 (114 days). *Presentation* anterior; *position of foetus* dorso-ilio-sacral.

Fig. 8 (119 days). *Presentation* posterior; *position of foetus* dorso-ilio-pubic.

Fig. 9 (140 days, taken at 9.15 a.m.). *Presentation* anterior; *position of foetus* dorso-ilio-pubic.

Fig. 10 (140 days, taken 3 p.m.). *Presentation* anterior; *position of foetus* dorso-pubic.

On the 150th day (2nd February, 1934), the female lamb 28959 was born.

It will be seen at once after studying the above figures that the "defined position" of Franck (quoted by De Bruin) or "very nearly constant position of Craig did not apply to the ewes in question. Apart from the alterations of the foetal position the presentation actually changed three times (Ewe 37782) and twice (Ewe 37931) respectively, as recorded by the X-ray photographs. How many times there were alterations in presentation during the intervals it is impossible to say There is therefore no doubt but that the situation of the foetus varies a great deal. The factors, however, underling the regulation of foetal situation are not clearly understood. In the circumstances it is perhaps helpful to compare the foetus *in utero* to a ship at anchor, which moves on the anchor as the currents and winds compel. In the foetus, however, there is in addition some factor which in the final stages brings about cephalic presentation.

The photographs are reduced approximately to one-sixth of the original and details are therefore not as distinct as in the X-ray negatives.

We are much obliged to Mr. T. Meyer for making the prints.

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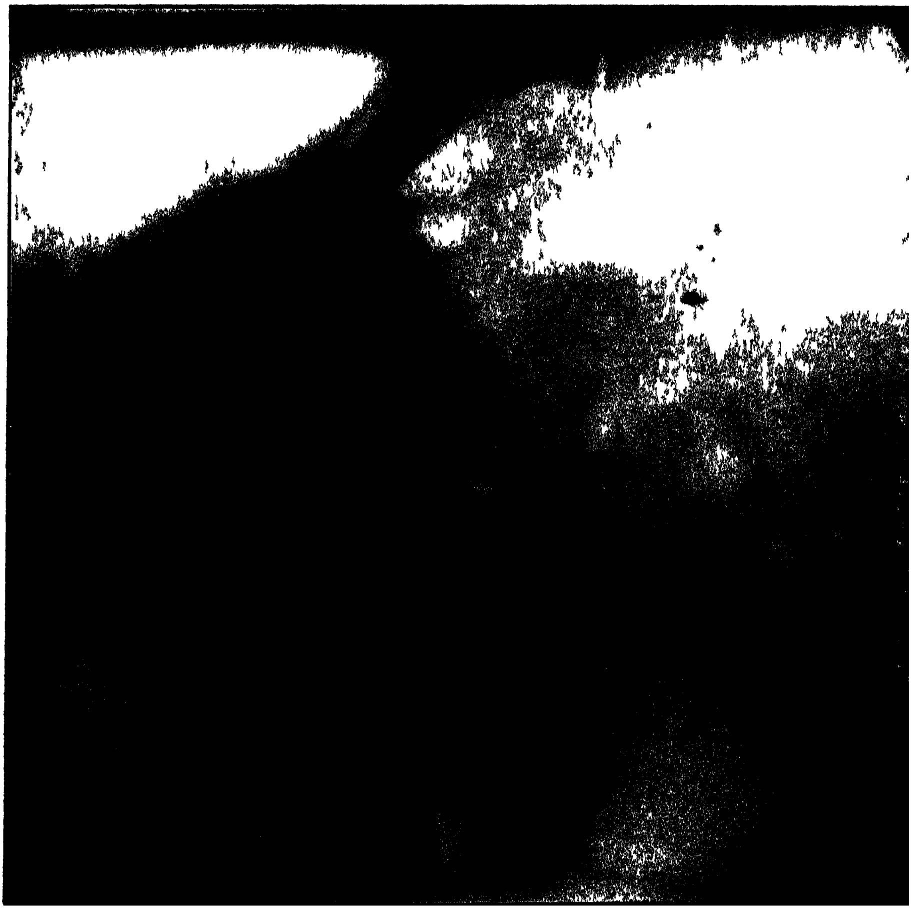


Fig. 1.

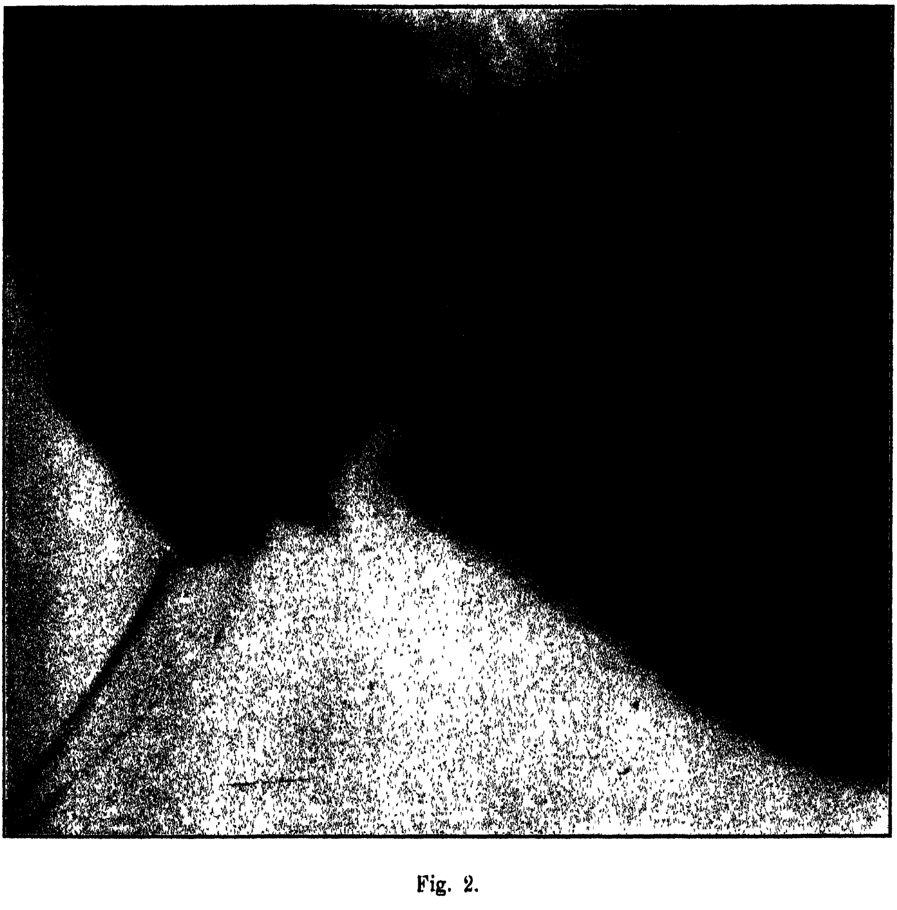
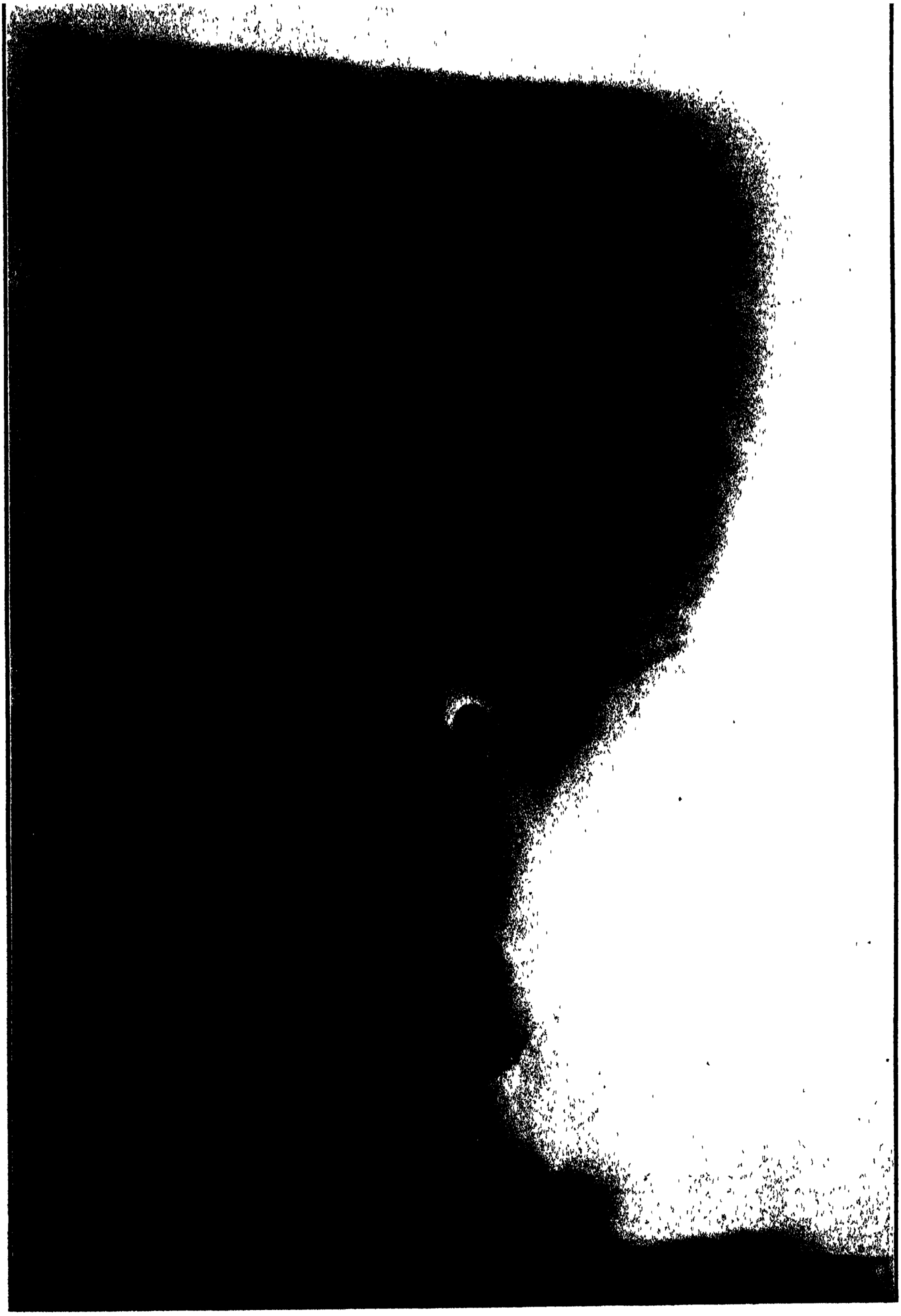


Fig. 2.



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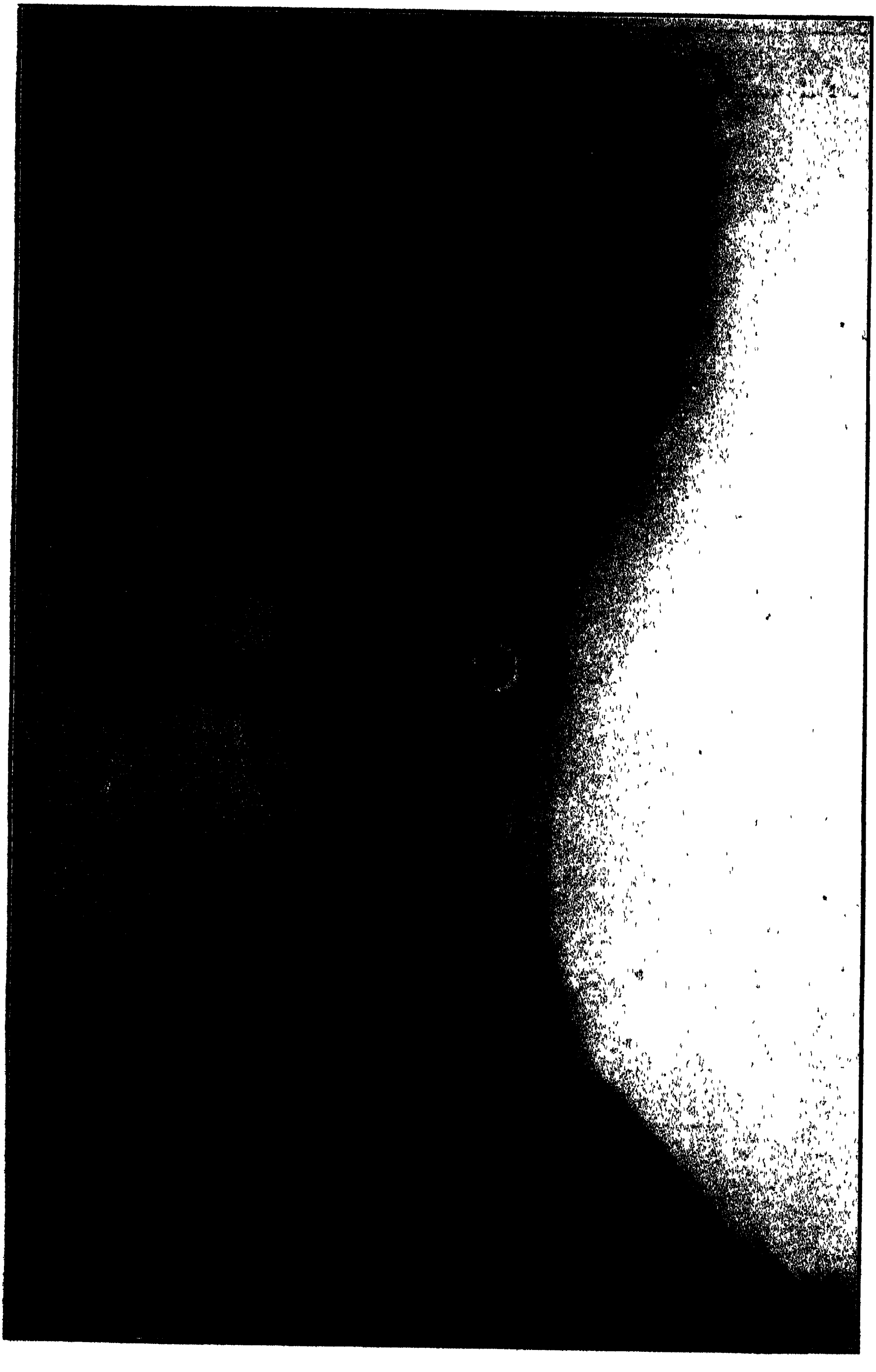


Fig. 4.

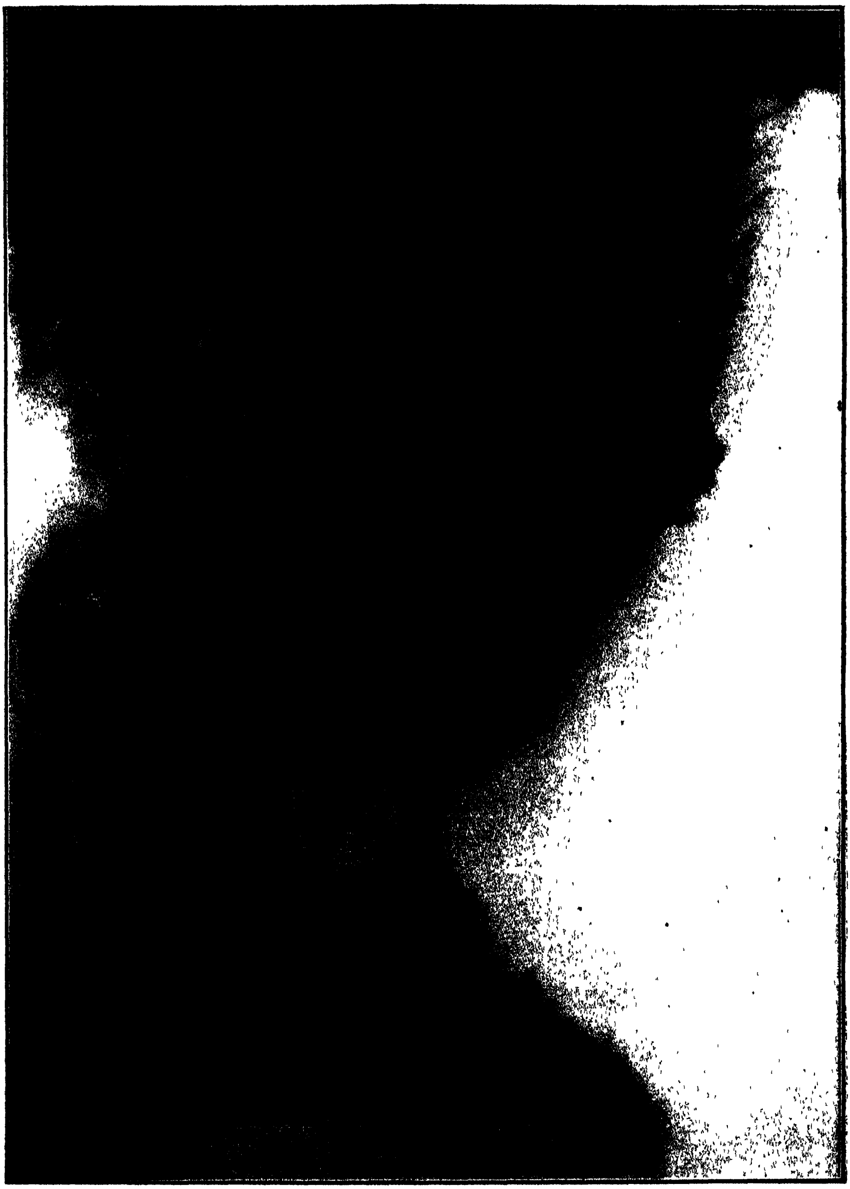
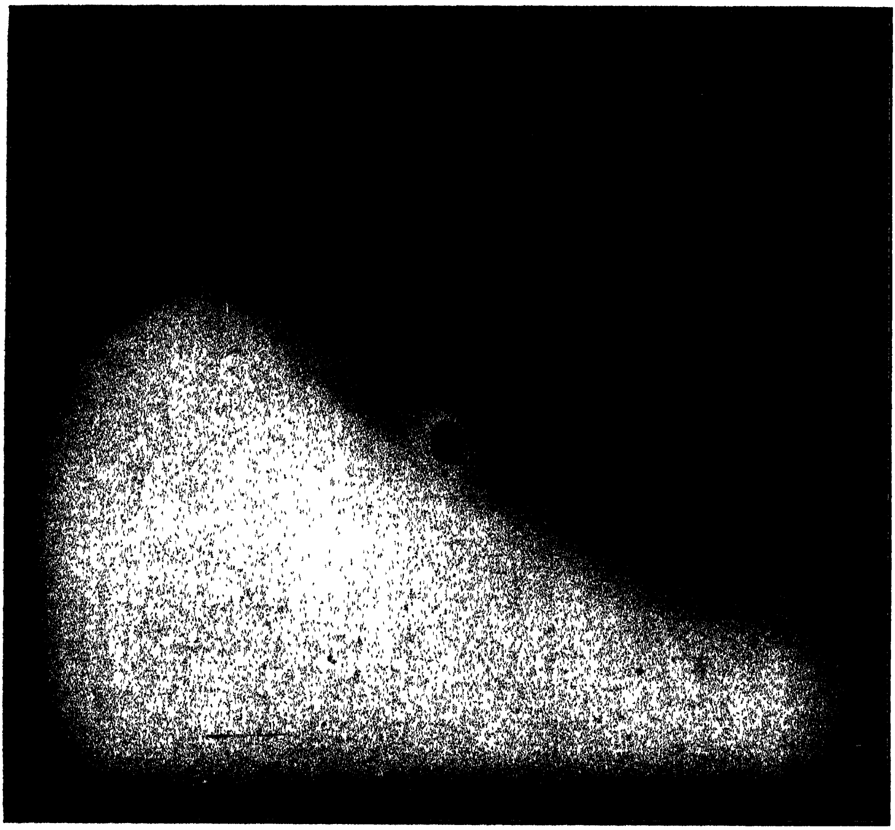


Fig. 5.



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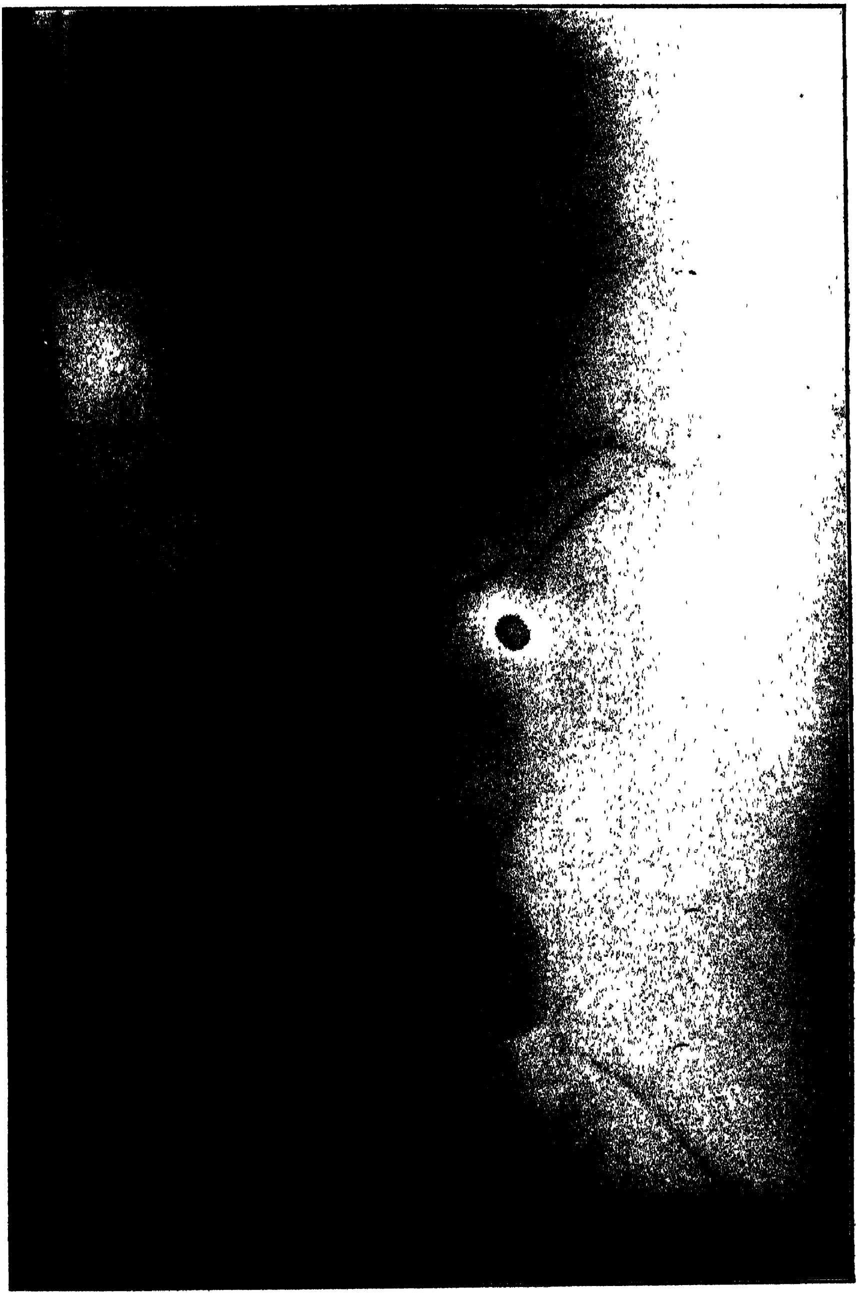

Fig. 7.

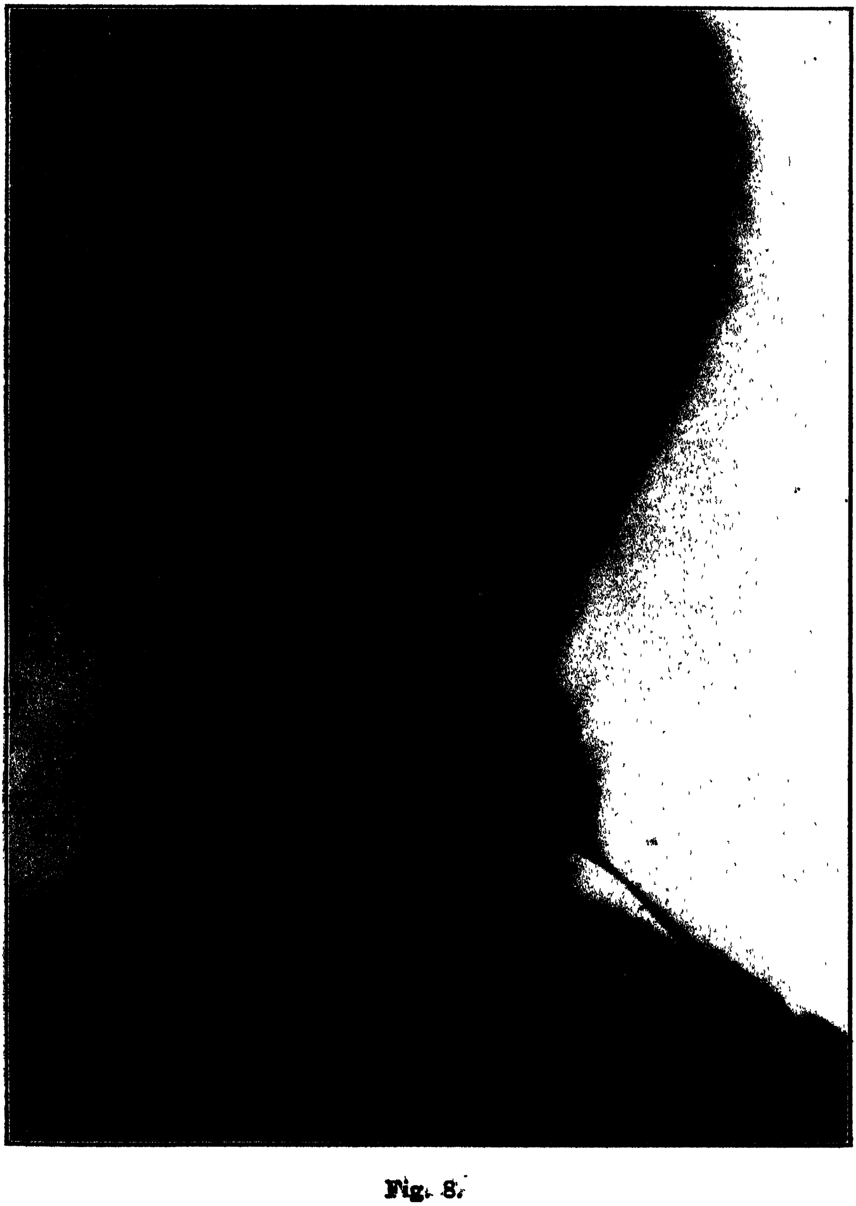

Fig. 8.

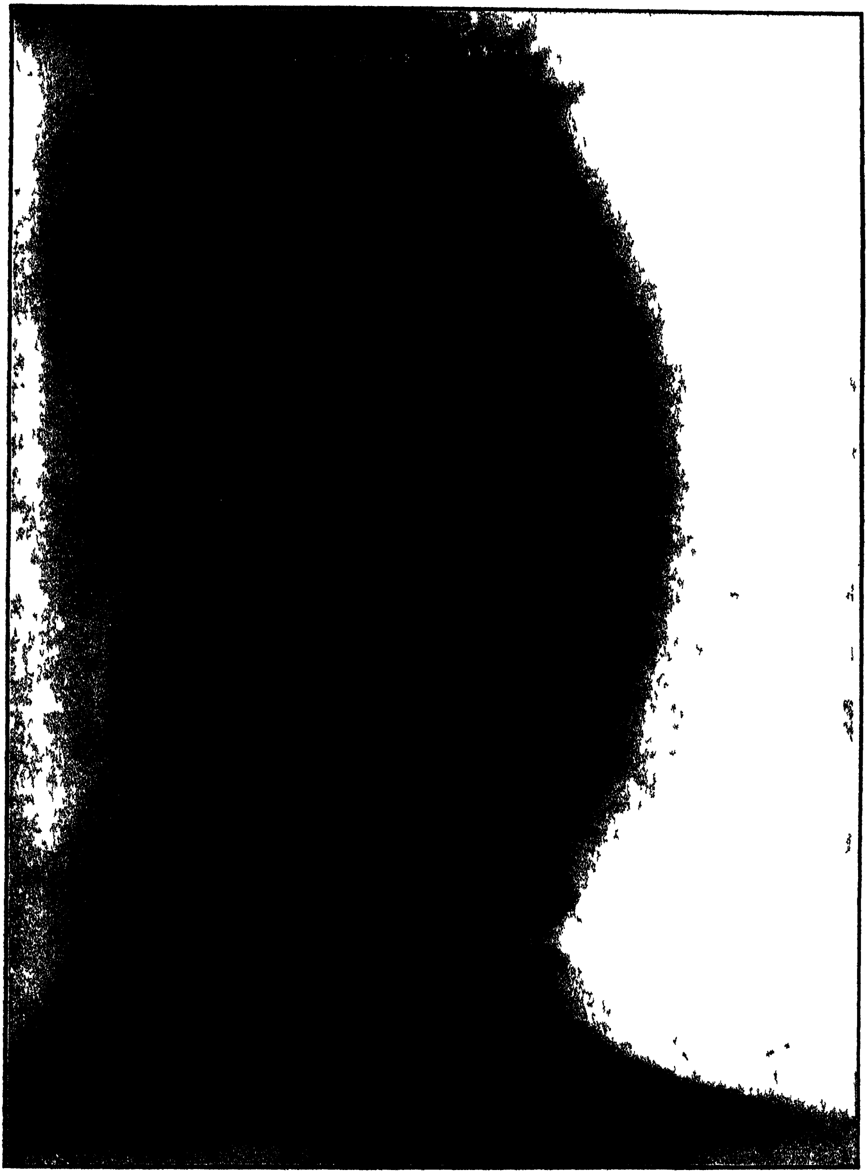

Fig. 9.

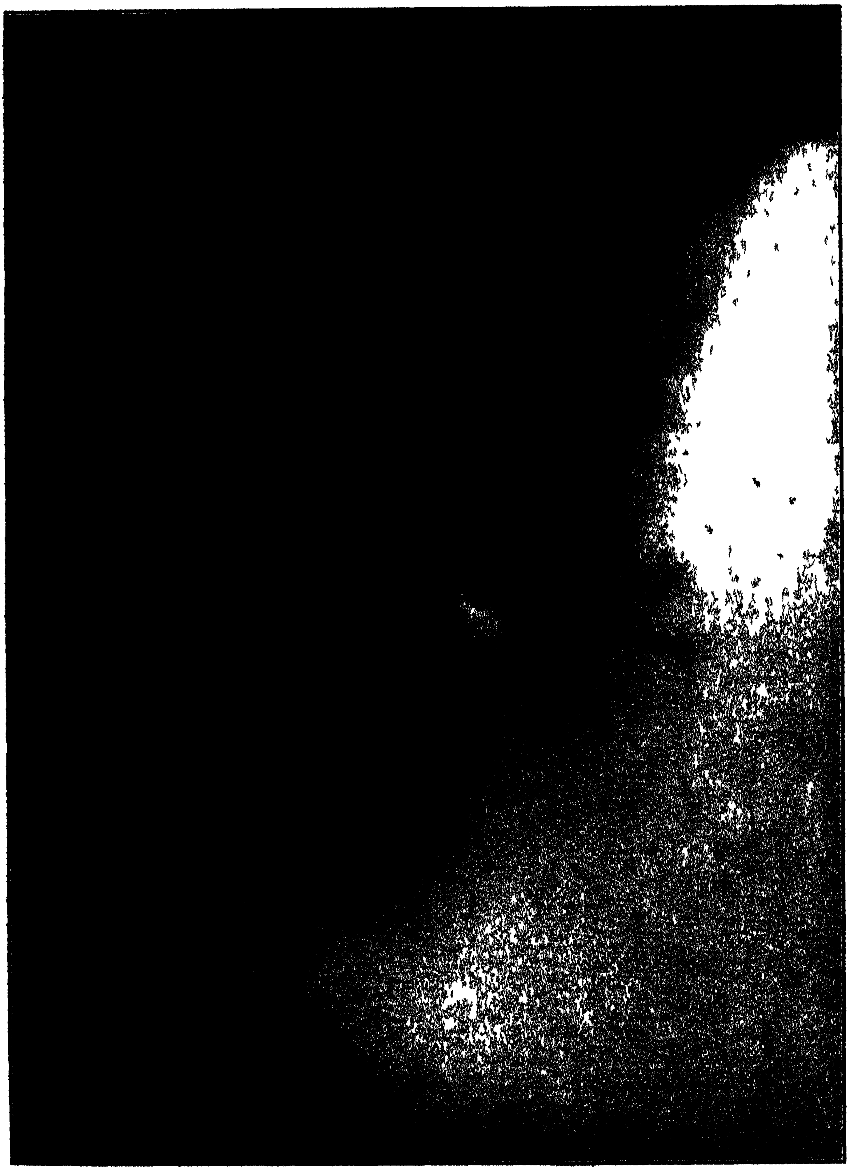

Fig. 10.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies in Sex Physiology, No. 15.

Further Observations on the Body Weight and Crown-Rump Length of Merino Foetuses.

By A. P. MALAN and H. H. CURSON, Sections of Statistics and Anatomy, Onderstepoort.

In Sex Physiology Study 13 (Curson and Malan, 1935), the effect of age on the weight and C.R. length of the Merino foetus was considered, observations being based on 13 foetuses originally referred to in Sex Physiology Study 10 (Foetuses 3, 10, 19, 22, 27, 33, 36 and 40) and Study 12 (Foetuses H, 42, 43, 44 and 45). The C.R. length was measured in a *straight* line, according to the method described by Keibel and Mall (1910).

In Study 13 an approximate relationship between body weight and C.R. length was given, this being based on an apparent constant ratio between the relative increases in weight and the corresponding increases in C.R. length. Since, however, only 13 foetuses were employed, the estimated relationship was inaccurate due to individual variation in foetal growth. In order, therefore, to improve on the position, more extensive data are used for the results which now appear in this paper.

The data in question are obtained from Sex Physiology Studies 10, 12 and 16(*). The one series of foetuses (mentioned in Studies 10 and 12) was obtained from the same flock, *i.e.* Grootfontein School of Agriculture, Cape Privince, while the other series (mentioned in Study 16) came from Ermelo, Transvaal. These data are tabulated below in Tables I and II.

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* See following Study which deals with the effect of age on the increase in surface area. For this a new series of foetuses was obtained.

TABLE I—(*The Grootfontein Series*).

	1 Foetus.	2 Age.		3 Weight. (gm.)	4 C.R. Length. (cm.)
		Days.	Hours.		
1....................	H	30	—	0·62	1·6
2....................	1	23	4	1·13	2·1
3....................	2	33	20	1·56	2·3
4....................	3	35	1	1·93	2·4
5....................	4	36	23	1·83	2·5
6....................	6	39	1	4·08	3·5
7....................	7	40	8	4·13	3·2
8....................	8	42	16	2·77	3·3
9....................	9	43	22	8·06	4·1
10....................	10	44	21	7·81	3·9
11....................	11	46	6	13 32	5 1
12....................	12	46	22	7·87	4 8
13....................	13	48	8	15 06	5·3
14....................	14	49	1	17 58	5 7
15....................	15	49	23	24 2	6·7
16....................	16	51	1	23 9	6·7
17....................	17	52	1	25·6	7·2
18....................	18	53	—	27·8	7·3
19....................	19	55	4	36·5	7·9
20....................	22	64	16	88 5	10 5
21....................	23	65	21	103 0	11 5
22....................	24	66	18	120	11 9
23....................	25	68	15	152	12·6
24....................	26	70	17	178	13·4
25....................	27	72	13	187	14 1
26....................	28	80	22	320	20·0
27....................	29	82	3	400	17·3
28....................	30	82	23	415	17·4
29....................	32	84	17	458	19·3
30....................	33	84	19	414	18 7
31....................	34	86	20	355	16·5
32....................	36	96	18	959	23·5
33....................	37	100	16	988	23·5
34....................	38	101	15	943	22·9
35....................	39	102	18	1,011	21·6
36....................	40	105	2	1,576	25·5
37....................	41	108	16	1,217	23
38....................	42	115	—	1,490	26
39....................	43	125	—	2,810	33
40....................	44	135	—	2,780	35
41....................	45	145	—	2,790	35

TABLE II—(*The Ermelo Series*).

	1 Foetus.	2 Age. (days.)	3 Weight. (gm.)	4 C.R. Length. (cm.)
1................	35,712	31	1	1·9
2................	35,510 Male	55	40	9·8
3................	35,659 „	61	70·6	11·0
4................	35,592 Female	64	82	12·9
5................	33,131 Male	92	617	25·0
6................	39,904 „	94	680	25·0
7................	32,969 „	107	1,320	29·5
8................	35,976 „	122	2,230	32·0
9................	45,060 „	147+2	2,975	40
10................	45,023 Female	+ 149	3,750	45

CHART A.

Comparison of Relative Increases in Body Weight and C.R. Length of the Merino Foetus.

(The straight line represents $y=0{\cdot}363x+0{\cdot}7255$)

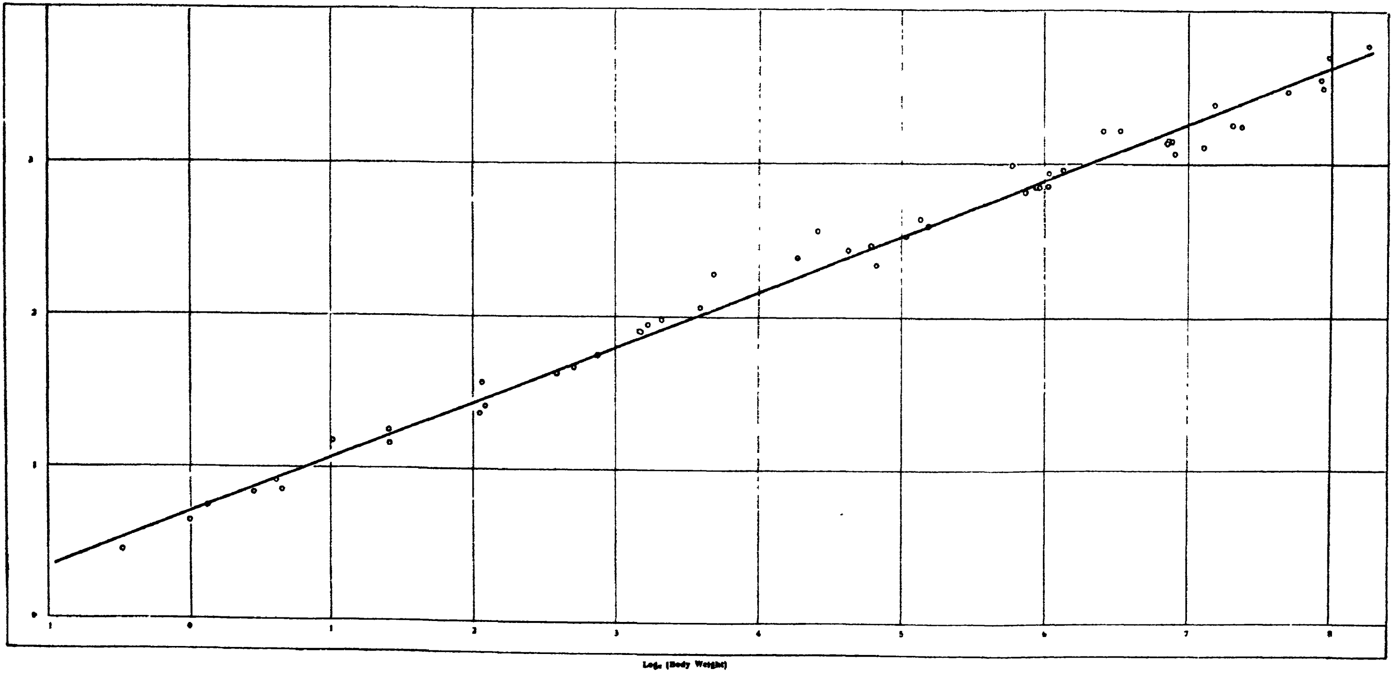
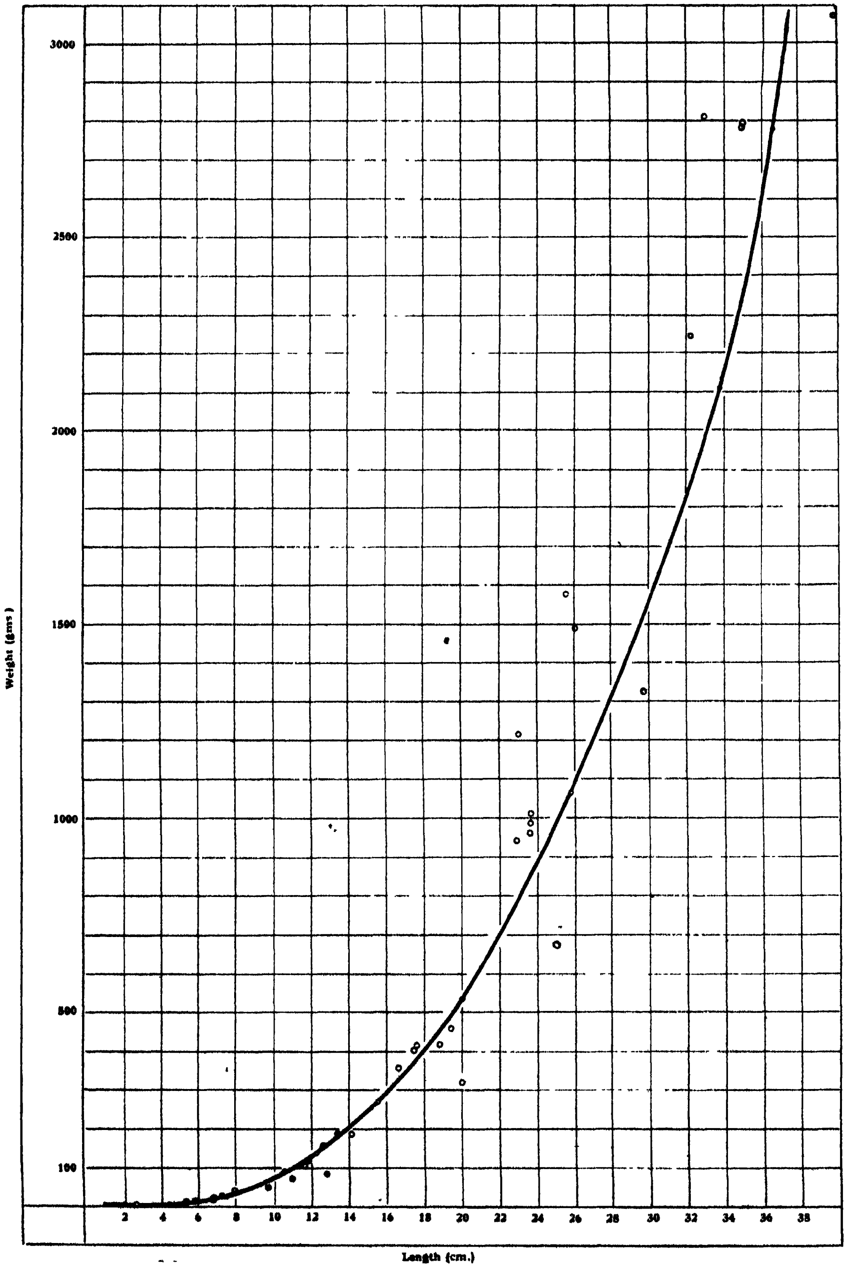


CHART B.

The Body Weight of the Merino Foetus plotted against its (straight) C.R. Length.

(The continuous line represents $w = 0 \cdot 147 l^{2 \cdot 785}$.)



When the natural logarithms of the weights and C.R. lengths are taken of all the data contained in Tables I and II, columns 3 and 4, and the values for the weight plotted against the corresponding values for length, a strong linear relationship is observed. This relationship is clearly illustrated by Chart A, where the continuous line represents the best fitting straight line.

The equation of this straight line was calculated from the logarithmic values by the method of least squares and is given by:—

$$y = 0{\cdot}363x + 0{\cdot}7255, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where $x = log_e$ (*weight*).

and $y = log_e$ (*C.R. length*).

As pointed out in the previous study (Curson and Malan, 1935) the linear relationship between the corresponding logarithmic values indicates a constant ratio between the relative increase in C.R. length and the corresponding relative increase in body weight. Or in other words, that the C.R. length, in units of actual observation, is proportional to a power of the body weight. This power index is represented by the " slope " of the straight line in Chart A and is equal to the coefficient of x in equation (1).

By converting equation (1) unto units of observation the following equation, illustrated in Chart B is obtained:—

$$l = 2{\cdot}066\ w^{0{\cdot}363} \quad \ldots\ldots\ldots \quad \ldots\ldots\ldots \quad (2)$$

where l = *C.R. length in cm.*,

and w = *body weight in gm.*

[It is evident that where in the above equations (1) and (2) the C.R. length measurements are expressed as a function of the body weight values, the converse, *i.e.* expressing weight as a function of C.R. length is equally possible. The corresponding equations are:—

$$x = 2{\cdot}759y - 1{\cdot}934 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)^1$$

and $w = 0{\cdot}147\ l^{2{\cdot}735} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)^1$]

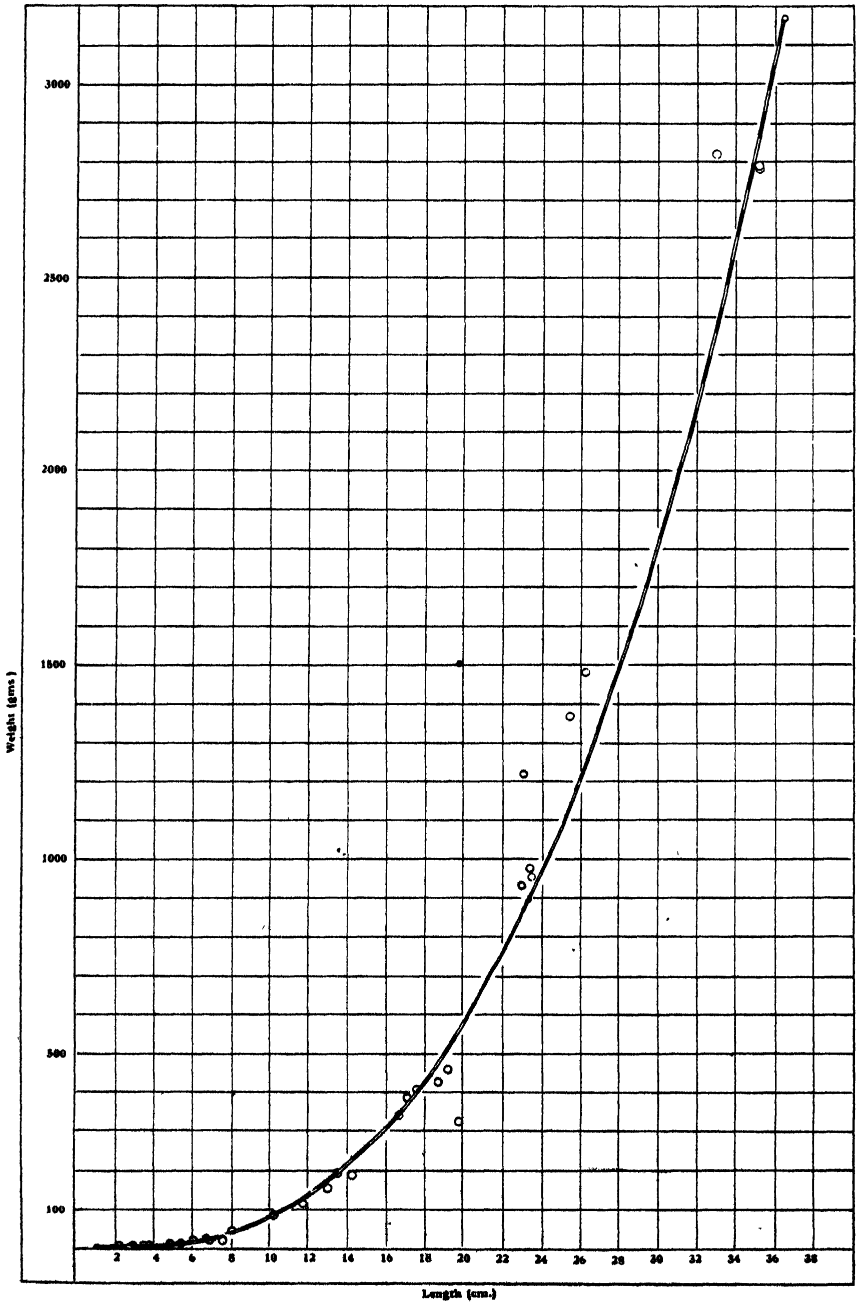
On the assumption of a linear relationship between the natural logarithmic values of weight and C.R. length it is clear that this relationship is uniquely determined by one point on the straight line (Chart A) and its slope. But to determine the accuracy, or standard error of the C.R. length, given by equation (1) for any particular natural logarithmic value of the body weight, one further assumption made in the calculation of this equation, must be adopted, *i.e.* the logarithmic values of all C.R. lengths during the gestation period had to be assumed as " equally variable ". This assumption also seems warranted by the data represented on Chart A.

When the deviation of a particular C.R. length from the corresponding *expected* value on the straight line given by equation (1) is considered, it has a standard deviation of which the estimate is $\pm 0{\cdot}0959$. These deviations, however, are differences between logarithmic values, and expressed in units of actual observation the above value is a ratio and should be interpreted as a standard deviation of 9·59 per cent. of the *expected* value.

CHART C. (GROOTFONTEIN SERIES.)

The Body Weight of the Merino Foetus plotted against its (straight) C.R. Length.

(The dotted line represents $w = 0 \cdot 135 l^{2 \cdot 80}$.)



The fact that the above estimate of the standard deviation is a constant percentage of the *expected* value, or in other words, that the standard deviation from the *expected* value expressed in units of observation is directly proportional to the corresponding *expected* value, is contained in the assumption made that the logarithmic values (of C.R. length and body weight) are equally variable.

[Taking the body weight as the dependent and C.R length as the independent variable and hence using equations (1)[1] and (2)[1] to estimate the standard deviation of an observed value for body weight, ±26·36 per cent is found.]

It is now proposed to briefly deal with the "slope" of the straight line which is given by the coefficient of the independent variable in equation (1) and also by the power index in equation (2). The standard error of the statistic is ±0·005328 and it is therefore *unlikely*† to obtain an estimate from a *corresponding* series of foetuses which will not fall between +0·350 and +0·377.

For the sake of comparison the data contained in Tables I and II were taken separately. The parabolic equations obtained from these series, together with the observed data, are graphically represented by Charts C and D. The equation for the Grootfontein series (Chart C) is:—

$$l=2\cdot07\ w^{0\cdot355}\ (\text{or } w=0\cdot135\ l^{2\cdot80})\ \ldots\ldots\ldots\ldots\ldots \quad (3)$$

and that for the Ermelo series (Chart D) is:—

$$l=2\cdot23\ w^{0\cdot366}\ (\text{or } w=0\cdot124\ l^{2\cdot70})\ \ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where as before,

w = body weight in gm.
and *l = C.R. length in cm.*

TABLE III.—(See Sex Physiology Study 13).

1 Foetus.	2 Age. (days.)	3 Weight. (gm.)	4 Curved C.R. Length (cm.). (observed.)	5 Curved C.R. Length (cm.). (expected.)
H............	**30**	0·62	1·7	1·9
3............	**35**	1·9	2·5	2·8
10............	**45**	7·8	6 0	4·9
19............	**55**	36·5	9·3	8·7
22............	**65**	88·4	12·0	12·2
27............	**73**	187	17·5	16·3
33............	**85**	414	22·8	22·1
36............	**97**	959	27·3	30·3
40............	**105**	1,576	34·0	36·8
42............	**115**	1,490	37·5	35·9
43............	**125**	2,810	43·8	45·8
44............	**135**	2,780	45·3	45·6
45............	**145**	2,790	47·5	45·7

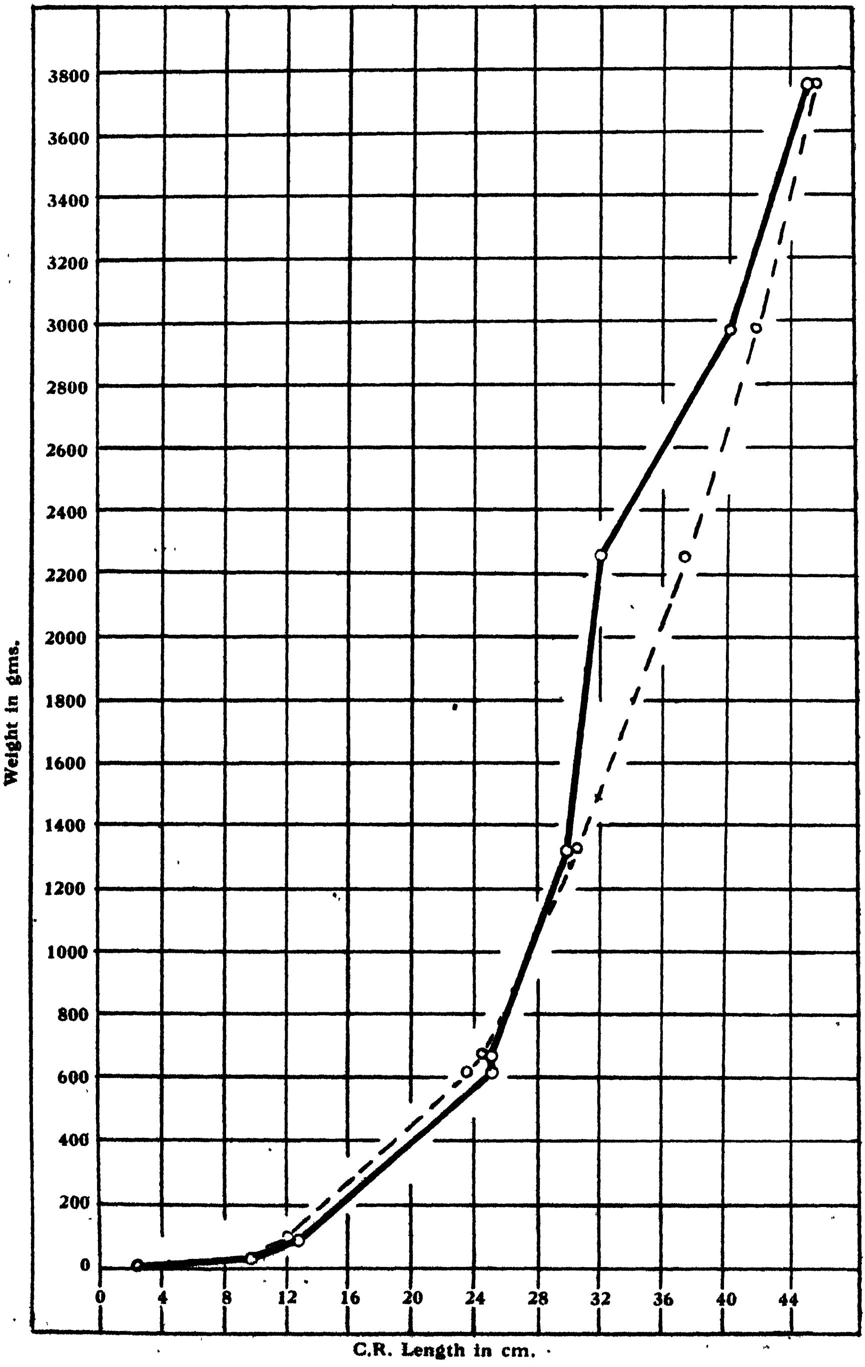
---

† **Note that *unlikely* refers to a probability less than 1 per cent.**

CHART D. (ERMELO SERIES.)

The Body Weight of the Merino Foetus plotted against its (straight) C.R. Length.

(Dotted line represents $w = 0{\cdot}124 l^{2{\cdot}70}$.)



As it was felt that the C.R. length measurements [given in the above tables and taken as indicated by Keibel and Mall (1910)], depend largely on the posture of the foetus, it was considered possible that a more reliable C.R. length might be obtained by measuring *along the back* instead of taking a straight line from the crown to the rump. Observations of the modified C.R. length were accordingly made, and the thirteen foetuses dealt with in Sex Physiology, No. 13 (Curson and Malan, 1935) were again measured, this time along the back and the results tabulated in Table III.

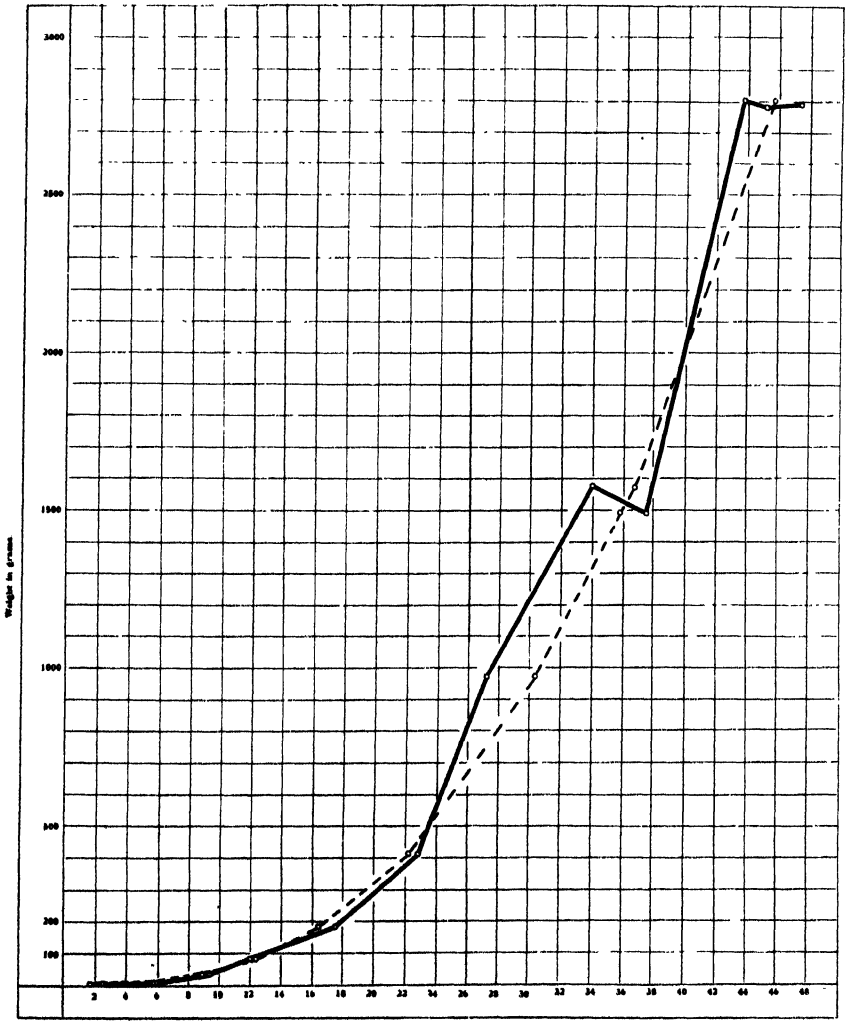
The relationship between the weight and "curved" C.R. length is given by the following equation:—

$$l = 2{\cdot}214\ w^{0\cdot382}\ (w = 0{\cdot}131\ l^{2\cdot60})\ \ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

CHART E.

The Body Weight of the Merino Foetus plotted against its (curved) C.R. Length.

(Dotted line represents $w = 0{\cdot}131 l^{2\cdot60}$.)



The discrepancies between the observed and expected C.R. lengths (Table III) are shown by the points on Chart E, joined by continuous and dotted lines, respectively. Comparing the results obtained here with those in Sex Physiology Study 13, Chart D, the "fit" does not appear to be improved. The data are however too few for a definite conclusion.

It is striking how little information is available regarding C.R. lengths. Needham (1931), in the appendix, giving the normal magnitudes in embryonic growth, gives (Vol. III, pp. 1669-1678), merely the length (except in Table 18 where C.R. length is stated). That this factor should receive more attention and definite details indicated is exemplified in the striking differences to be noted in the length of certain measurements by Galpin (1935) and Curson and Quinlan (1934). The values given by Galpin appear to correspond with the curved C.R. length measurements of Table III, column 4. A comparative table is given hereunder:—

TABLE IV.

Age. (Days.)	Massey Agricultural College.	Onderstepoort.	
		Straight C.R. Length.	'Curved' C.R. Length.
42	6 cm.	3·3	—
43	—	—	6
55	—	—	11
56	11	8·4 (twins)	—
64	14	10·5	—
65	—	—	12
68	15	12·6	—
72	19–20	14·1	—
73	—	—	17·5
83	22–23	18·5 (twins)	—
85	—	—	22·8

SUMMARY.

1. The relationship between C.R. length and weight of two series of foetuses from Grootfontein School of Agriculture, Cape Province, and Ermelo, Transvaal, is considered. The results given below are clearly illustrated by the charts in the text.

(*a*) To series combined:—$l = 2{\cdot}066\ w^{0{\cdot}363}$ ($w = 0{\cdot}145\ l^{2{\cdot}735}$).

(*b*) Grootfontein series:—$l = 2{\cdot}07\ w^{0{\cdot}355}$ ($w = 0{\cdot}135\ l^{2{\cdot}80}$).

(*c*) Ermelo series:—$l = 2{\cdot}228\ w^{0{\cdot}366}$ ($w = 0{\cdot}124\ l^{2{\cdot}70}$).

2. The thirteen foetuses used in Sex Physiology Study No. 13, which is part of the Grootfontein series, were employed to determine

a relationship between weight and C.R. length along the curvature of the back. The result is illustrated by Chart E. The equations for the relationships under discussion are:—

(*a*) Straight C.R. length:—

$l = 1{\cdot}97\ w^{0{\cdot}361}\ (w = 0{\cdot}154\ l^{2{\cdot}77})$.

(*b*) "Curved" C.R. length:—

$l = 2{\cdot}214\ w^{0{\cdot}382}\ (w = 0{\cdot}134\ l^{2{\cdot}60})$.

3. It is suggested that for uniformity more attention be paid towards adopting a definite length measurement as a standard for studies in foetal growth.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies in Sex Physiology, No. 16.

Surface Area in the Foetus of the Merino Sheep.

By A. P. MALAN and H. H. CURSON, Sections of Statistics and Anatomy, Onderstepoort.

INTRODUCTION.

In reality this study is a continuation of Study 13 (Curson and Malan) where the effect of age was studied in relation to (*a*) general body form, (*b*) body weight, and (*c*) C.R. length. As the foetuses in question were required for the further investigation of the external body form, another series of foetuses has been obtained through the kindness of Dr. L. L. Roux, M.Sc., of Ermelo, for this observation.

Neither Needham (1931) nor Hammond (1932) has any information bearing directly on the subject.

As the ordinary method of removing the skin and calculating its area on a measuring table was obviously unsatisfactory, we adopted the suggestion of Mr. F. E. H. Appleton of Onderstepoort. This consisted in dipping the foetus in a celloidin solution, allowing the material to dry, removal of the celloidin coat and then measuring this. In this way was obviated the stretching of the skin which would nullify the results obtained.

In Foetuses 32969 (107 days) and 35976 (122 days) and Lamb 45060 (two days plus gestation period 147 days), since the hairy coat was well established, no attempt was made to remove separately the adhering celloidin coat, which in any case, prevented stretching of the skin during removal.

The data obtained from the ten foetuses are tabulated thus:—

TABLE I.

1 Lamb.	2 Age. (Days.)	3 (*w*) Weight. (gm.)	4 (*l*) C.R. Length. (cm.)	5 (*a*) (2) Surface Area. (sq. cm.) (Observed.)	6 (2) Surface Area. (sq. cm.) (Expected.)
No number.......	± 28	—	—	3·1	—
35712............	31	1	1·9	3·5	3·6
38510............	55	40	9·8	58	58·3
35659............	61	70·6	11·0	90	90·9
35592............	64	82	12·9	98	101·6
33131............	92	617	25·0	440	485
39904............	94	680	25·0	522	524
32969............	107	1,320	29·5	854	875
35976............	122	2,230	32·0	1,275	1,314
45060............	147 + 2	2,975	40·0 (1)	1,920	1,644
45023............	± 149	3,750	45·0	2,100	1,992

(1) Height at withers on second day was 34·5 cm.

(2) The legs below the carpus and tarsus were not taken into consideration.

DISCUSSION.

We shall deal now with the effect of age on (*a*) the increase in surface area and (*b*) the relation between surface area and body weight.

(*a*) *The Effect of Age on the Increase in Surface Area.*

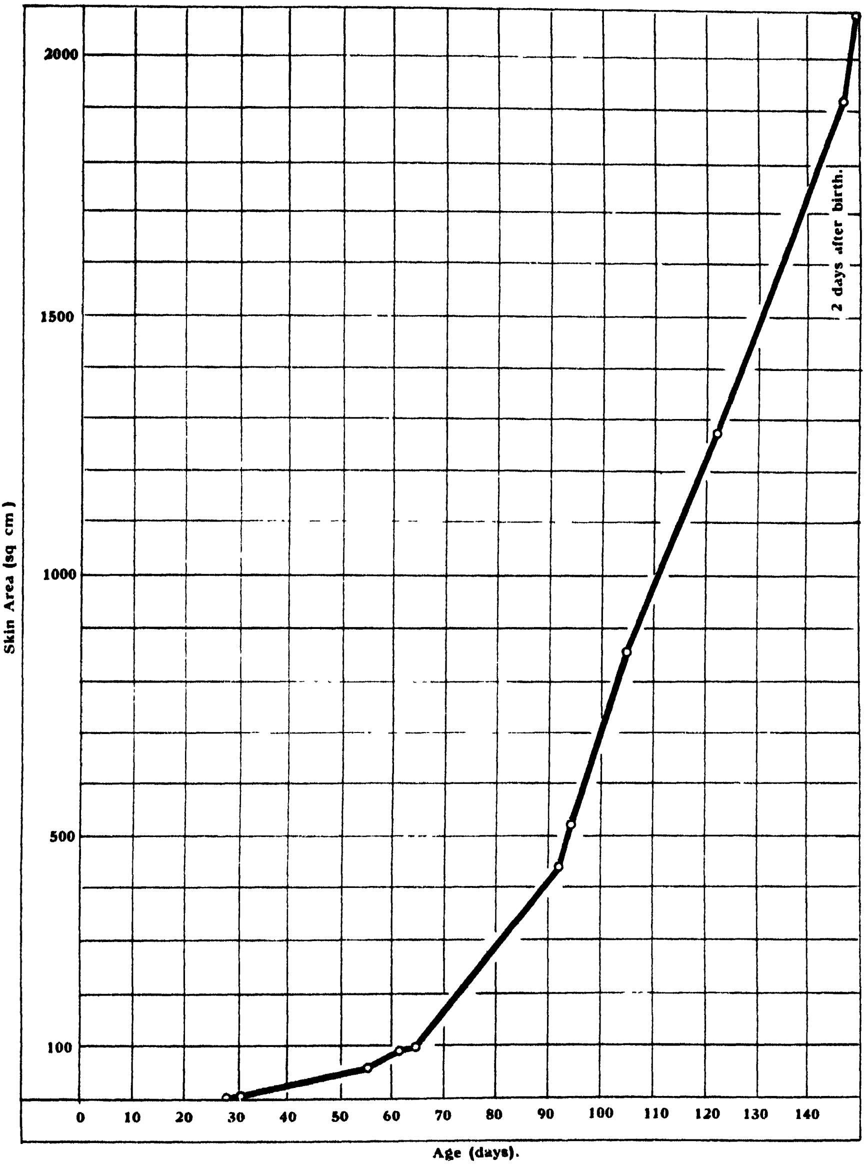
The information available concerning skin area at different ages is contained in columns 5 and 2 respectively in Table I. These particulars are diagramatically shown in Chart A, where surface area is plotted against age and the various points joined by a series of straight lines.

As in other features of prenatal growth, *e.g.* body weight and C.R. length, the increase in surface area with age is exceedingly slow during early pregnancy, but increased rapidly with advancing age. This acceleration in the growth rate has its maximum in the beginning of the gestation period and decreases to a negligible value towards the end.

The average rate of increase in surface area for the whole prenatal period was approximately 13 sq. cm. *per diem*. How this rate of increase, however, varies with age is shown in Chart A, and also if one arbitrarily divides the gestation period into three stages of about fifty days each. The growth rates in surface area during these successive intervals are approximately 1 sq. cm. *per diem*, 15 sq. cm. *per diem*, and 25 sq. cm. *per diem* respectively. It is also obvious that the total increase during the first half of pregnancy is very little in comparison with that during the second half.

CHART A.

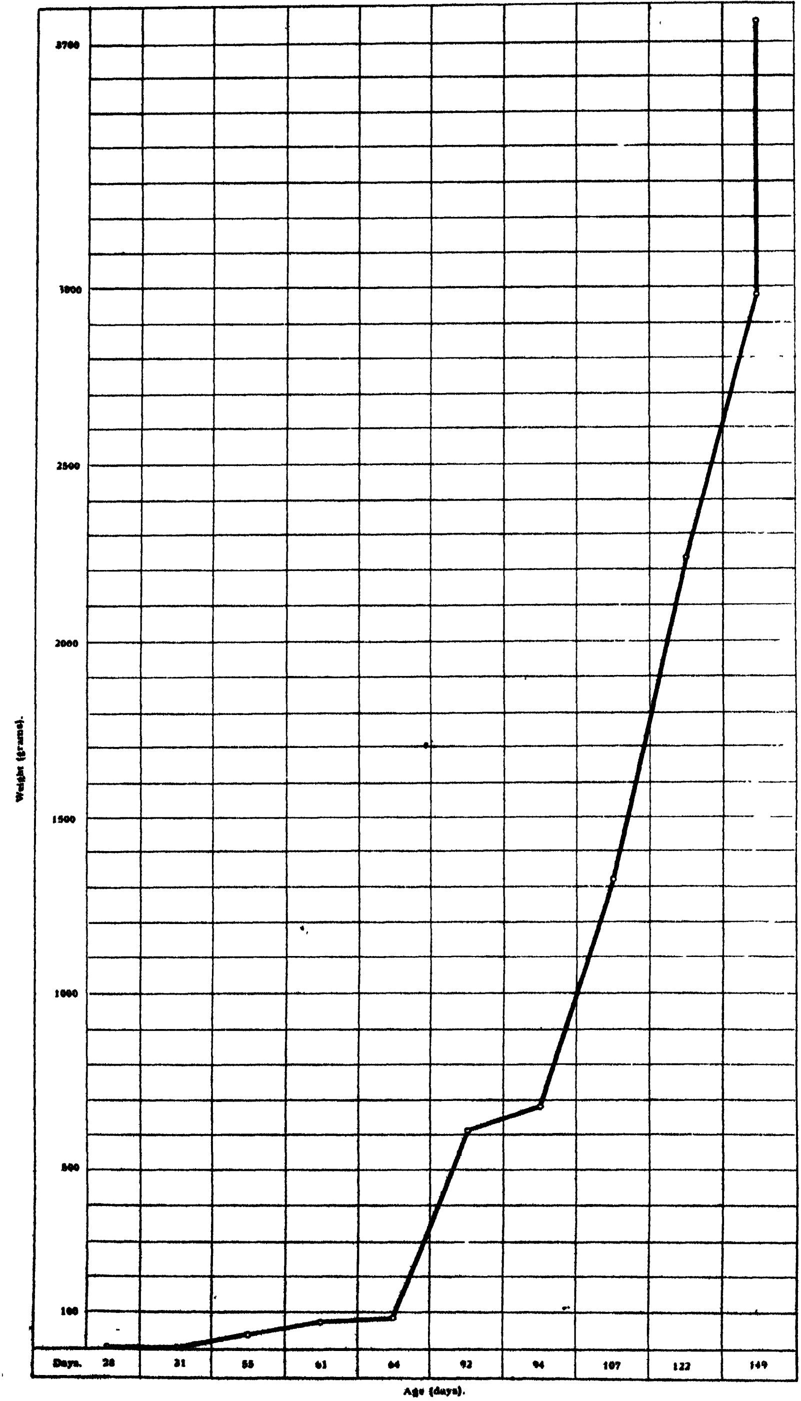
The Increase of the Skin Area of the Merino Foetus.



The surface area is found to double itself during the last forty days of the gestation period, and the skin area of the mature Merino sheep (Lines and Pierce) is approximately five and a half times that of the new born lamb. (The body weight also doubles itself during the last forty days of the gestation period, *but* the mature weight is approximately twelve times the birth weight.)

CHART B.

The Growth in Body Weight of the Merino Foetus.



(b) *The Effect of Age on the Relation between Surface Area and Body Weight.*

For the sake of comparison with Chart A (which illustrates the increase of surface area with advancing age), Chart B has been prepared to show the growth in body weight for the same series of foetuses. There is a remarkable resemblance between the two graphs except for a less marked increase of body weight during the first half of pregnancy and a more pronounced growth in weight during the second half. This indicates the relatively greater acceleration of the growth in body weight.

A better picture, however, of the actual relationship is furnished by Chart C where $\log_e$ (weight) is plotted against $\log_e$ (surface area) as tabulated below.

CHART C.

The Relation between the Relative Growths in Weight and Skin Area for the Merino Foetus.

(Dotted line: $x = 1{\cdot}284y - 1{\cdot}530$.)

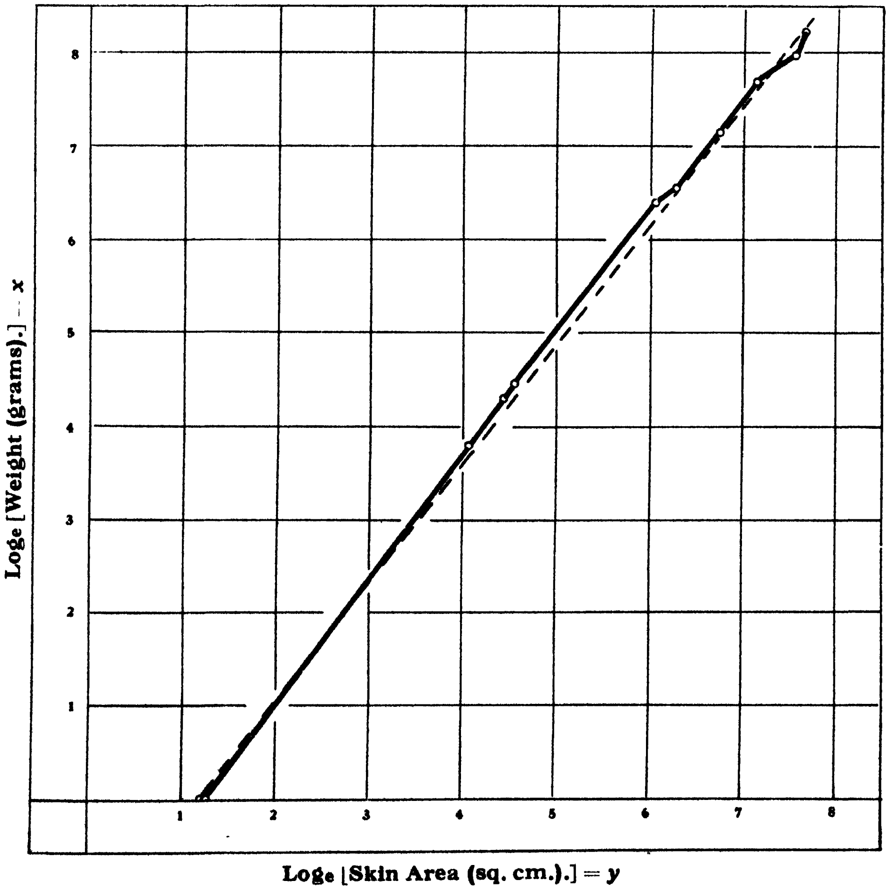


TABLE II (*Logarithmic values*).

Lamb.	Log$_e$ (C.R. Length).	Log$_e$ (Weight). x	Log$_e$ (Skin Area). y
No number	—	—	1·1314
35721	0·6419	—	1·2528
38510	2·2824	3·6888	4·0604
35659	2·3979	4·2627	4·4427
35592	2·5649	4·4067	4·5850
33131	3·2189	6·4249	6·0868
39904	3·2189	6·5221	6·2558
32969	3·3843	7·1854	6·7499
35976	3·4657	7·7098	7·1507
45060	3·6888	7·9980	7·5596
45023	3·8067	8·2295	7·6497

The strong linear relationship between the logarithmic values is clearly shown by Chart C. This apparent linearity suggests a constant ratio between the relative increases in surface area and those in body weight. $\left\{ \frac{d}{dt} (log_e w) = \frac{1}{w} \frac{dw}{dt} \right.$, and $\frac{d}{dt} (log_e a) = \frac{1}{a} \frac{da}{dt}$. Therefore for a constant relationship between these relative increases, a straight line is to follow when the natural logarithms of the weight are plotted against the natural logarithms of the *corresponding* skin areas. $\}$ The best fitting straight line to these logarithmic values was obtained by the method of least squares and is shown on Chart C by the dotted line, its equation being:—

$$x = 1{\cdot}284y - 1{\cdot}530 \text{ or } y = 0{\cdot}778x + 1{\cdot}200 \quad . \; . \; . \; . \quad (1)$$

where $x = log_e$ (*weight*) and

$y = log_e$ (*area*) as shown in Table II.

When in the above equations the logarithmic values are transformed into units of actual observation the following parabolic relationships are obtained:—

$$w = 0{\cdot}217a^{1{\cdot}284} \text{ or } a = 3{\cdot}320w^{0{\cdot}778} \quad . \; . \; . \; . \; . \; . \; . \; . \quad (2)$$

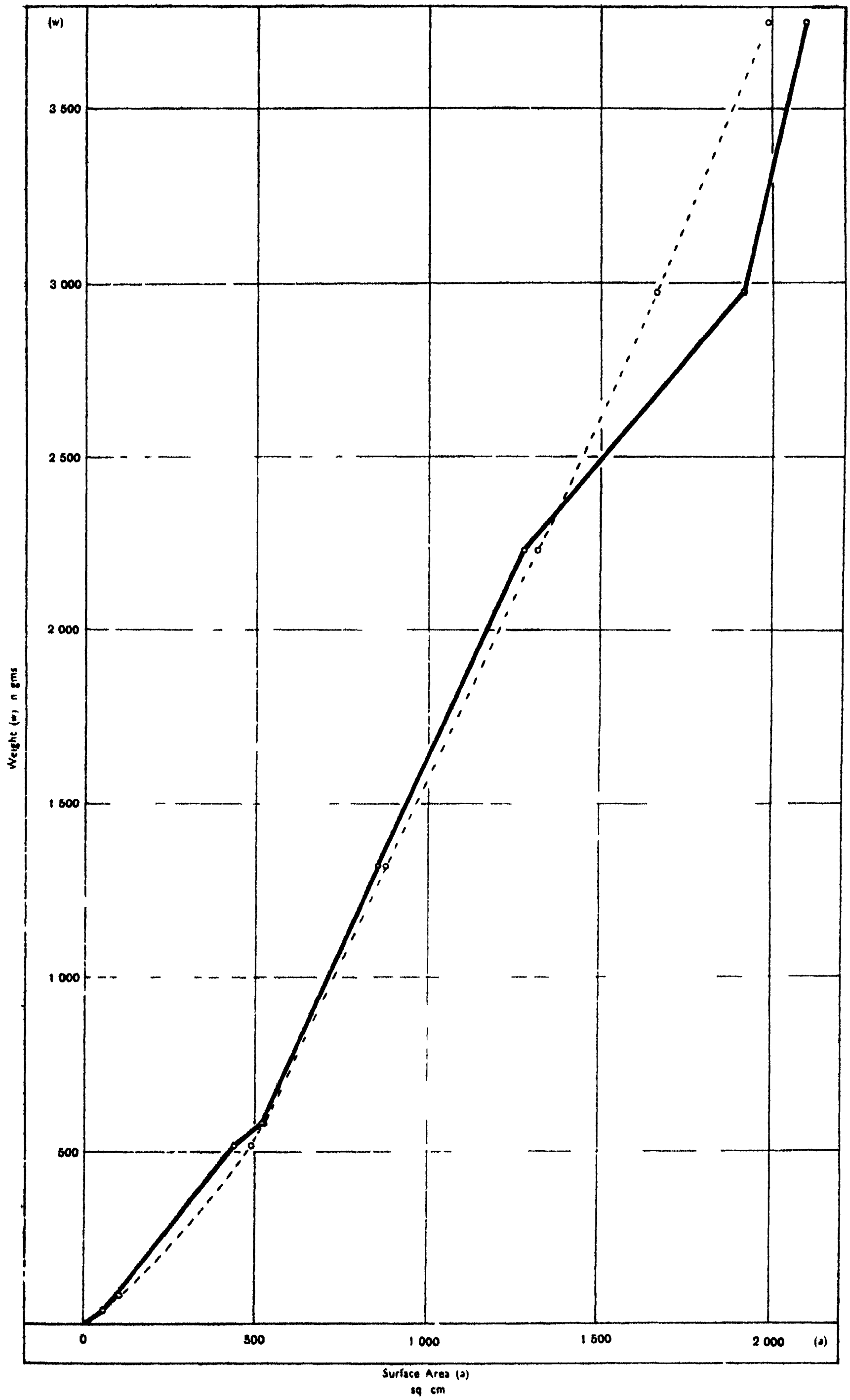
where w = *body weight in gm.* and

a = *surface area in sq. cm.*

This relationship is shown by the dotted line on Chart D. The points joined by a series of continuous straight lines represent the observed values for body weight and surface area, as given in columns 3 and 5 of Table I, while the expected surface areas for the corresponding observed weights are calculated from the above relationship (2) and given in column 6 of Table I. These "expected" values are in fair agreement with those observed. Although the obvious discrepancy of the second last point in chart D is, when expressed as a percentage, not unduly great, some explanation may be given. Lamb 45060 was measured two days after birth and the fact that it was rather thin explains the relatively low weight in comparison with skin area.

CHART D

The Relation between Body-weight and Surface-area of the Merino-foetus.



In various publications on basic metabolism of sheep (*e.g.* Lines and Pierce, 1931), different values for the constants in the above relationship (2) are given.* A fairly exhaustive review of the literature on the subject is given by Brody (1934) in the *Annual Review of Biochemistry.* The power index in the parabolic relationship between surface area and weight is $\frac{2}{3}$ according to Meeh, whose value is most frequently adopted. Brody, however, suggests 0·70 which is still rather lower than the value for the prenatal stage as shown above.

Although the number of foetuses for which data concerning both weight and skin area exist is only ten, the value for the index is given by the present method of calculation as 0·778±0·0104. This value may therefore not be expected for 1 per cent. probability from an actual value which lies outside the limits 0·75 and 0·81. It is, however, suggested that this value may vary from breed to breed and even differ slightly for different flocks of the same breed.

Another point for consideration was the relationship between surface area and C.R. length and the relationship between surface area and both weight and C.R. length. Since, however, surface area was more accurately expressed in terms of body weight than in terms of C.R. length, and furthermore since the remaining variance in surface area which was not expressed in terms of body weight was of the same order as the accuracy of observation, the inclusion of C.R. length in the present case serves no useful purpose.

SUMMARY.

The increase in surface area of the Merino foetus is discussed.

The relationship between surface area and body weight, as estimated from the present series of foetuses is expressed by the equation $a = 3{\cdot}320\ w^{0{\cdot}776}$.

Several charts are given to illustrate the features described.

ACKNOWLEDGMENT.

We wish to thank Messrs. C. G. Walker and F. D. Horwell for their assistance and interest in the observations.

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* See Section "Heat-production of Mammalian Embryos", p. 732, Vol. II, Needham.

ADDENDUM.

In addition to the observations presented and discussed in the preceding article the following information is now available:—

TABLE.

Foetus of Ewe.	Age of Foetus (days).	(w) Weight in gm.	(l) C.R. length (cm.).	(a) Surface area (sq. cm.).	
				Observed.	Expected.
44803............	33	1·25	2·4	4·4	4·6
44849............	60	44·5	10·5	79	71
15337............	90	530	24	451	472
35821............	121	2170	36	1353	1387
44397............	145	3300	37	1930	1911

The data given in the above table should be compared with Table I in the text. (The straight C.R. length is included merely for the sake of completeness.)

The "expected" values for the surface area in the last column of the above table are calculated from the following equations.

$$y = 0{\cdot}765x + 1{\cdot}358 \quad (x = 1{\cdot}307y - 1{\cdot}771),$$

where $y = \log_e$ (surface area),

and $x = \log_e$ (body weight).

These equations are obtained by fitting straight lines to the data in the third and 5th columns.

When these equations are transformed to units of actual observation the following parabolic equations are obtained:—

$$a = 3{\cdot}889 \; w^{0{\cdot}765} \quad (w = 0{\cdot}170 \; a^{1{\cdot}307}).$$

where a = *surface area in sq. cm.*

w = *body weight in gm.*

The agreement between the observed and expected values is obvious.

The numerical coefficients in the above equations are by no means significantly different from those given in the text. Therefore, in order to obtain more reliable estimates of these coefficients all the relevant data given in both Table I (already referred to) and the above table were taken together. The resulting parabolic equations are:—

$$a = 3{\cdot}543 \; w^{0{\cdot}771} \quad (\text{and } w = 0{\cdot}196 \; a^{1{\cdot}295}).$$

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies in Sex Physiology, No. 17.

The Extent of the Gravid Merino Uterus in Relation to the Vertebral Column in the Dorsal Recumbent Position and the Weights of the Gravid Uterus and Foetus in Relation to the General Body Weight.

By H. H. CURSON and A. P. MALAN, Sections of Statistics and Anatomy, Onderstepoort.

INTRODUCTION.

THE observations made in connection with the relationship of the gravid uterus to the vertebral column are of academic interest, in that during life the uterus, which occupies a ventral abdominal position is separated from the vertebral column by the stomach and intestines It is nevertheless instructive to note the increasing area " covered "(*) by the uterus of the various intervals of pregnancy, particularly the cranial limit of the developing organ.

Of greater interest is the ratio not only between the weight of the gravid uterus to the general body weight (minus the weight of the unopened uterus); but also the relationship between the foetal weight and the general body weight of the mother (also minus the weight of the unopened uterus).

LITERATURE.

Works on obstetrics, *e.g.* Craig (1930) and Wyman (1901), naturally refer to the usual relations of the enlarging uterus. Craig (p. 104) states that " in ruminants the gravid uterus passes into the abdomen and extends between the right sac of the rumen and the abdominal wall ". Wyman adds (p. 7) that " the anterior free end not supported by the broad ligaments is covered by the great omentum ".

---

* i.e. When the ewe is in the dorsal recumbent position.

The photographs accompanying our Study unfortunately illustrate only the increase of size of the uterus in relation to the lumbar and thoracic vertebrae. Hammond (1932), while not concerned with the position of the uterus in the abdominal cavity, gives useful information regarding the lumbar region of sheep. He indicates (*a*) in Table LVII (p. 216) that the lumbar formula of his animals was usually 6, and mentions (*b*) that " the variability which exists in the lumbar and also of the thoracic vertebrae . . . shows the possibility of increasing the comparative length of this part by selection ". He adds (*c*) that " it appears probable . . . that the seat of greatest post-natal growth occurred at the end of the thoracic and beginning of the lumbar region ". Nathusius (quoted by Hammond, p. 219) states that the actual length of the lumbar region is independent of the number of lumbar veitebrae, but as Hammond points out, he does not give the relative length of lumbar region to other parts in the sheep under consideration.

In regard to growth in foetal weight there is but little information available. In fact, Needham (1931) states that " Colin is the only investigator who has ever determined " this factor. The figure he reproduces (Fig. 39, p. 379, Vol. 1) shows the weight as a percentage of the system occupied by the embryo, foetal membranes and foetal fluids and not as a percentage of the weight of the ewe.

OUR OBSERVATIONS.

(*a*) Sisson (1917, p. 156) gives the vertebral formula of the sheep as C7, T13, L6-7, S4 and Cy 16-18, but our experience with the Merino shows that L 7 is more common than L6. Also in the adult, owing to the practice of docking lambs, the number of coccygeal segments remaining is less than half the normal number.

The details bearing on the relationship referred to in the title are summarised in Table I.

The increase in volume of the uterus may be judged by perusal of Figs. 1, 3, 5, 8, 11, and 13; but it may be added that whereas the greatest depth of the non-gravid uterus is approximately 3 cm., and 10 cm. after one month's pregnancy, this figure has increased to 15 cm. at full term.

It must be emphasised that the body of the uterus is exceedingly short (about 2-3 cm.) but owing to the posterior part of the horns being united by connective tissue and having a common peritoneal lining, a false idea may be obtained as to the extent of the body.

(*b*) From columns (2) and (4) have been calculated the percentage weight of the intact or gravid uterus to the general body weight.

The weights of the unopened uterus, expressed as a percentage of the weight of the ewe (less weight of unopened uterus) [Table I, column (6)], are represented graphically on the accompanying chart. It is to be noted that the values in Table I, columns (4) and (6), marked with an asterisk, are approximately double the value to be expected from a comparison of the whole series.

TABLE I.

(1) Number of ewe.	(2) Live weight of ewe. (Kg.)	(3) Stage of gestation. (Days.)	(4) Weight of unopened uterus (Gm.) (ii)	(5) Weight of ewe less weight of uterus unopened. (Kg.)	(6) Weight of unopened uterus as a percentage of the weight of the ewe (iii). (%)	(7) Weight of foetus as a percentage of the weight of the ewe (iii). (%)	(8) Number of lumbar vertebrae.	(9) Pre-sacral vertebrae 'covered' by uterus.
38584.......	38·56	Non-gravid	158	—	—	—	7	2 (see fig. 2).
25924.......	38·56	3	163	38·39	0·4	0·00	7	2 (see fig. 4).
35712.......	27·44	31	165	27·82	0·6	0·00	7	2
38510.......	38·78	55	870	37·91	2·3	0·11	—	—
35659.......	34·13	61	2,000*	32·13	6·2*	0·22	6	7 (see fig. 6).
35592.......	31·75	64	1,440*	29·93	4·8*	0·27	7	7 (see fig. 7).
33131.......	33·57	92	2,090	31·47	6·6	1·96	7	11 (see fig. 9).
39904.......	39·46	94	2,155	37·31	5·8	1·82	7	9½ (see fig. 10).
32969.......	38·10	107	2,982	35·12	8·5	3·76	—	—
35976.......	35·38	122	3,380	32·00	10·6	6·97	7	12 (see fig. 12).
45023.......	42·86	±149	3,700	37·16	15·3	10·09	7	12

(ii) This includes vulva vagina, fallopian tubes and ovaries. In Study 12, column 4, the heading obviously should read "Approximate total weight of unopened uterus." There the weight included the oviducts and ovaries.

(iii) Less weight of unopened or gravid uterus.

From earlier observations (Sex Physiology, Study 10) it appears that the weight of the unopened uterus (Ewe 180) at a gestation age of 64 days is 1,000 gm.,* in which case the weight of the unopened uterus is approximately 3·25 per cent. of the weight of the ewe. This value, 3·25 per cent., is also shown on the chart by a square dot and evidently lies very near the expected value.

Ignoring the values for 61 and 64 days, given in Table I, column (6) and marked with an asterisk, a smooth curve has been drawn through the data, its equation being:—

$$y = 0{\cdot}00131\, x^{1{\cdot}875},$$

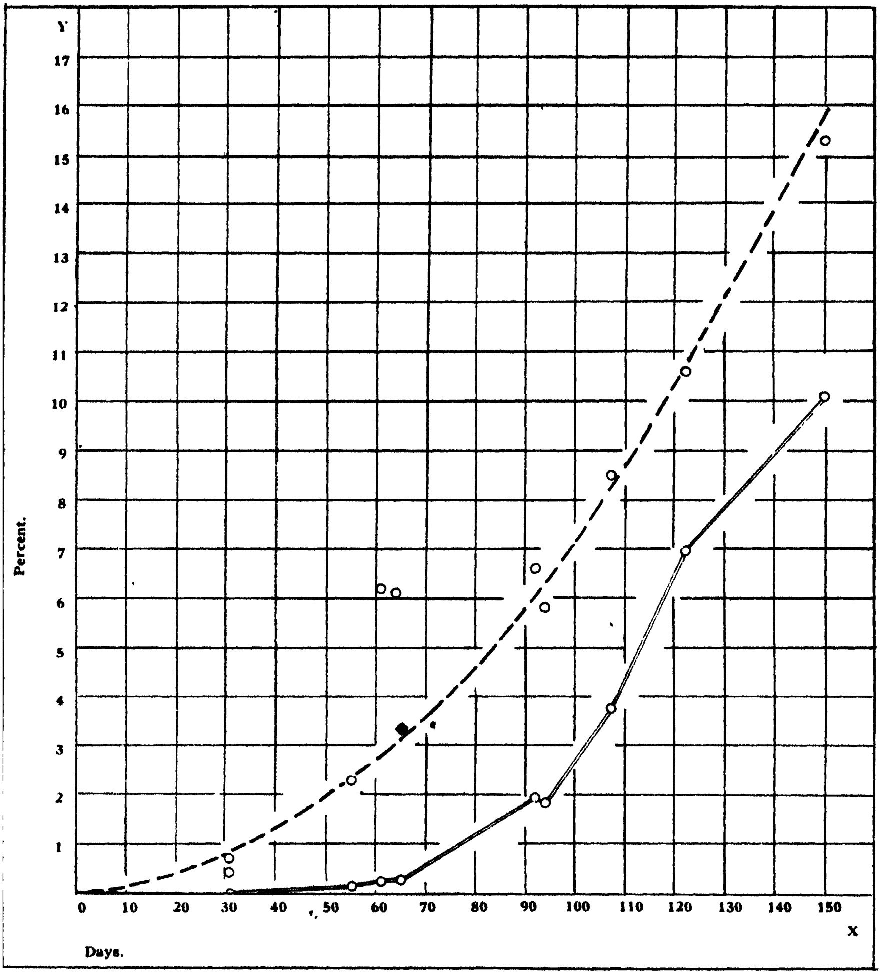
where x = *gestation period in days*,

$$y = 100 \times \frac{\textit{weight of unopened uterus,}}{\textit{weight of the ewe-wt. of the unopened uterus.}}$$

* Subsequently (3.2.36) ewe 30169 was killed on 61st day of pregnancy and the weight of the unopened uterus was 1,192 gm.

CHART 1.

Weight of (*a*) the unopened uterus and (*b*) the foetus as a precentage of the weight of the ewe.



The weight of the foetus, expressed as a percentage of the ewe weight [Table I column (7)] is also represented on the chart. These points are joined by a series of *straight* lines. It is to be observed that the foetus weights for 61 and 64 days show no such irregularity as was observed in the weights of the unopened uterus. This strengthens the conclusion that the discrepancies mentioned are more likely due to an error than to individual variation.

SUMMARY.

The increasing extent of the gravid uterus is well illustrated in the Figs. 1, 3, 5, 8, 11, and 13, and the relationship [see column (9) of Table I] to the vertebral axis in Figs. 2, 4, 6, 7, 9, 10 and 12.

The weights of the unopened uterus and of the foetus alone are expressed as percentages of the ewe weight (minus unopened uterus). Details are set down in columns (6) and (7) of Table I and a diagrammatic representation is given in the chart.

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ADDENDUM.

Further to the data recorded above, subsequent observations have been made as follows:—

(*a*) The extent of the vertebral axis " covered " by the gravid uterus (the ewe being in the dorsal recumbent position) at the various stages of pregnancy is shown in the following tabulated summary:—

No. of Ewe.	*Stage of Gestation.*	*Presacral vertebrae " covered " by uterus.*
35894	32 days	4
44803	33 "	4
45082	60 "	6
44849	60 "	6
15337	90 "	11
21665	90 "	9
44679	121 "	14
38521	121 "	14
44397	145 "	13
30514	146 "	14

In these observations, as in the former series, the cranial limit of the uterus was determined *after* the stomach and intestines had been removed. Obviously if determined *before* removal of the viscera, the cranial limit in advanced pregnancies would not have been so far anterior owing to the influence of the interposing organs.

As would be expected there is a general agreement between the obcervations recorded here and in the former series.

(*b*) In regard to the relationship between the (i) gravid uterus and (ii) foetus, and the ewe weight less weight of gravid uterus expressed as a percentage, the additional data, *i.e.* obtained from the ewes above, will be included in a further discussion on the subject in Sex Physiology Study 18.

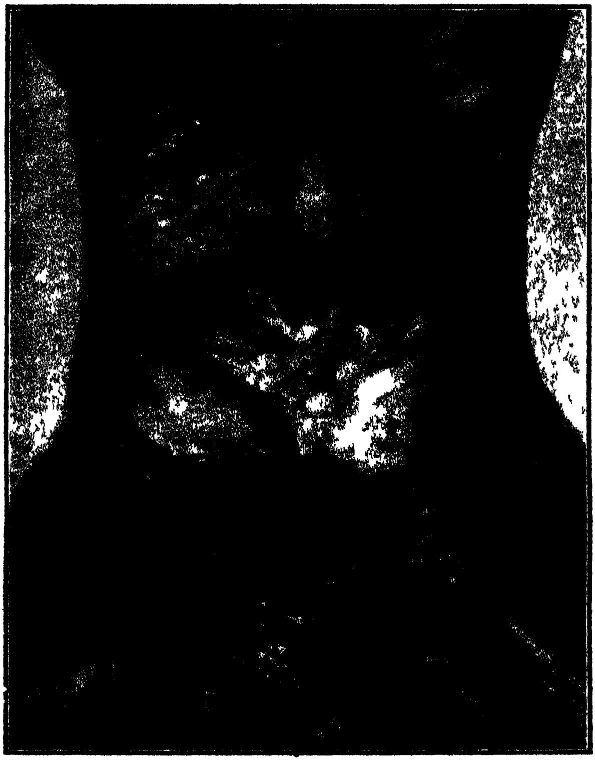

Fig 1.—Ewe 38548, non-pregnant Ventral view of uterus. (±¼ of original.)

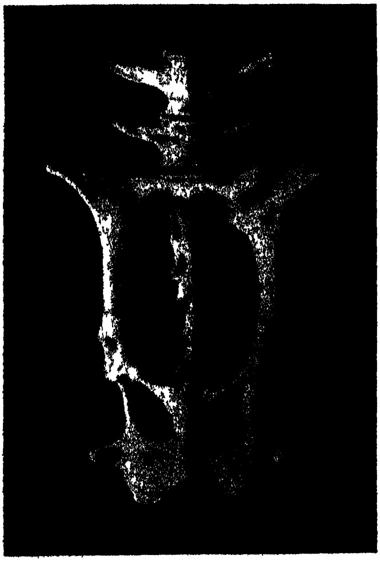

Fig 2.—Bony pelvis of Ewe 38548, non-pregnant. The uterus extends over 2 presacral vertebrae. (±¼ of original.)

Fig 3.—Ewe 25924, pregnant 31 days Ventral view of gravid uterus, right horn pregnant. (±¼ of original.)

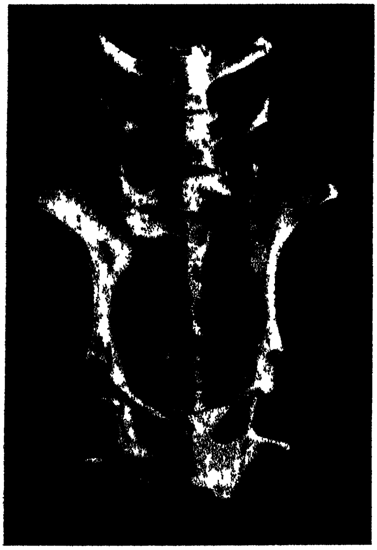

Fig. 4.—Ewe 25924. The 31 days pregnant uterus extends over 2 presacral vertebrae. (±¼ of original.)

Fig. 5.—Ewe 35659, pregnant 61 days. Ventral view of gravid uterus, left horn pregnant. (±¼ of original.)

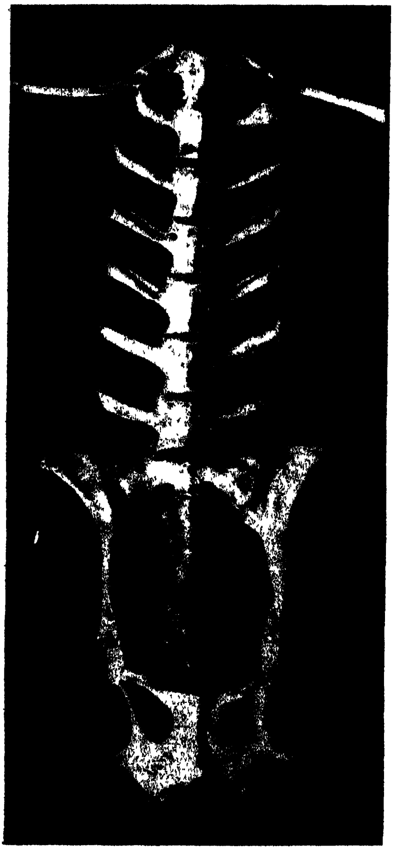

Fig. 6.

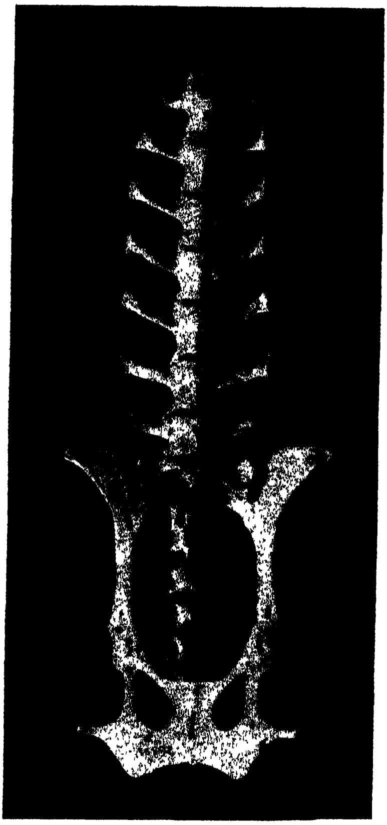

Fig. 7.

Fig. 6.—Ewe 35659. The 61 days pregnant uterus extends over 7 presacral vertebrae. (±¼ of original.)

Fig. 7.—Ewe 35592. The 2 months (64 days) pregnant uterus extends over 7 presacral vertebrae. (±¼ of original.)

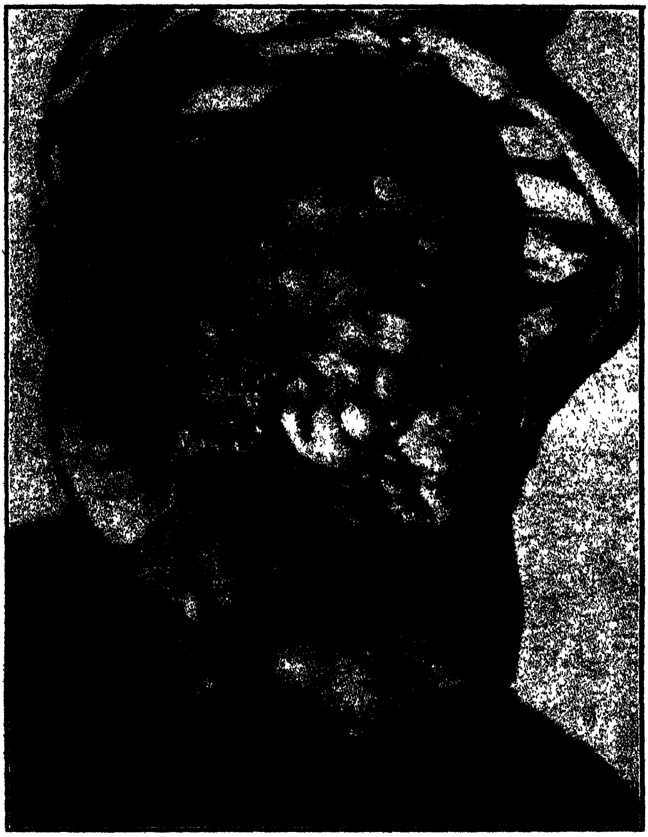

Fig. 8.—Ewe 33131, pregnant 92 days. Ventral view of gravid uterus, right horn pregnant. (±½ of original.)

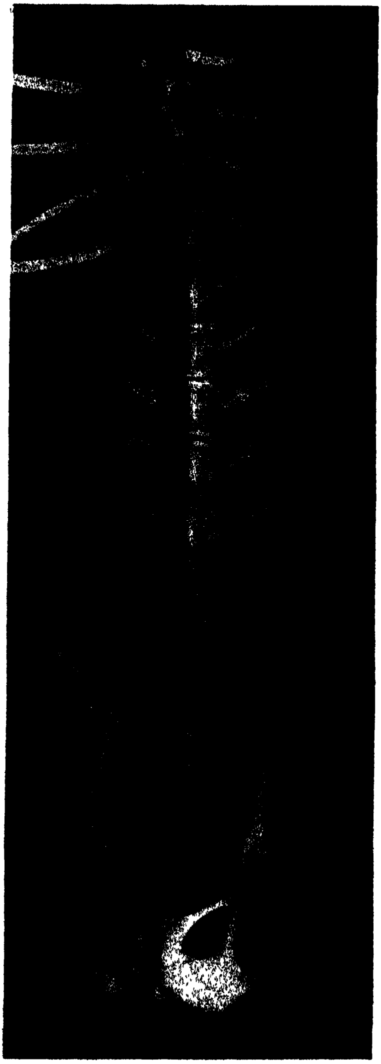

Fig. 9.

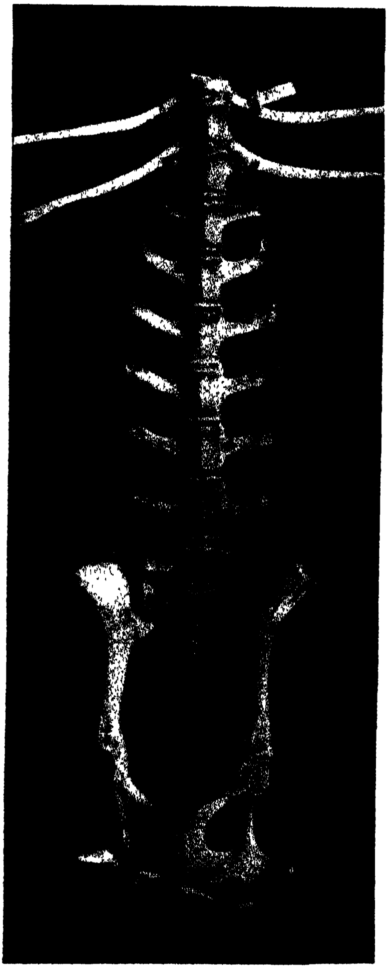

Fig. 10.

Fig. 9.—Ewe 33131. The 92 days pregnant uterus extends over 11 presacral vertebrae. (±¼ of original.)

Fig. 10.—Ewe 39904. The 3 months pregnant uterus extends over 9½ presacral vertebrae. (±¼ of original.)

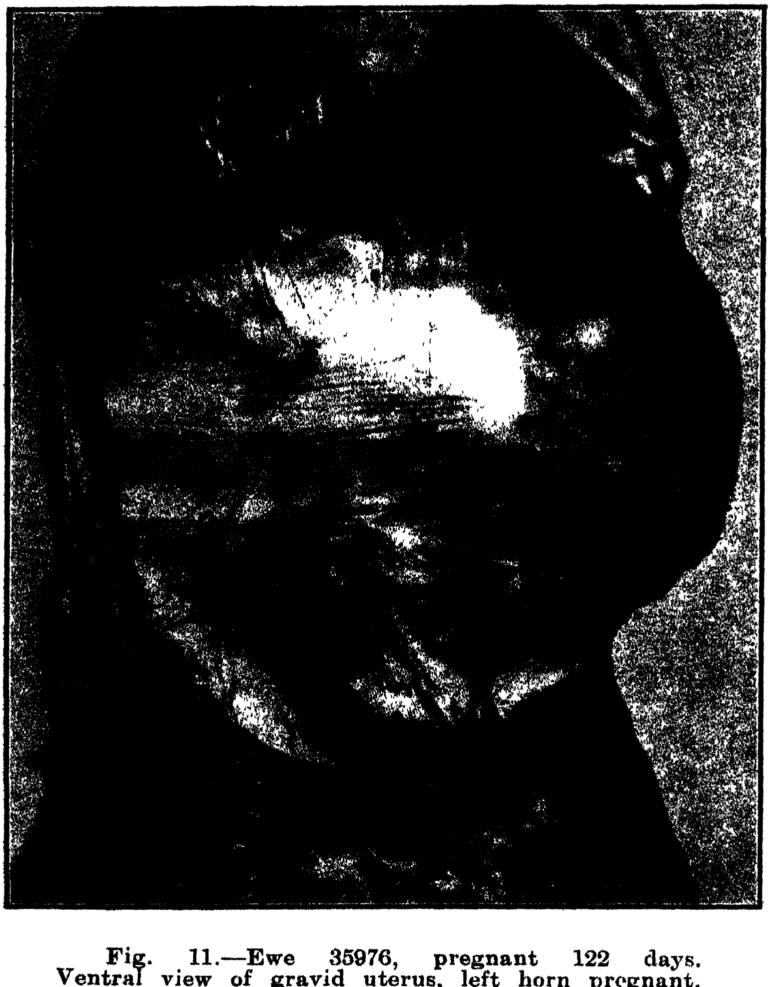

Fig. 11.—Ewe 35976, pregnant 122 days. Ventral view of gravid uterus, left horn pregnant. (±¼ of original.)

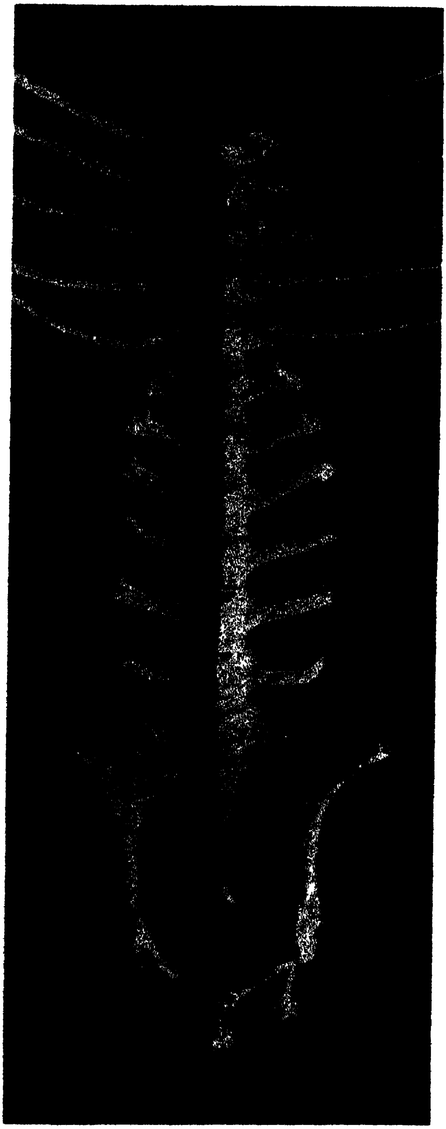

Fig. 12.—Ewe 35976. The four months pregnant uterus extends over 12 presacral vertebrae. (±¼ of original.)

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

A Contribution to the Study of the Pathology of Oesophagostomiasis in Sheep.

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Onderstepoort.

INTRODUCTION.

OESOPHAGOSTOMIASIS is perhaps the most serious parasitic disease of sheep in the Union of South Africa at the present time. A true understanding of the lesions produced by these parasites and especially a clear conception of the pathogenesis of the lesions may assist in evolving simple and efficient measures of control of the parasite, which is making sheep farming very difficult in many parts of the Union.

Weinberg (1909) gives a detailed microscopic description of twenty-three cases of oesophagostomiasis in various species of apes. The lesions of these apes infested with different species of oesophagostomum are all essentially the same. The author believes that the larvae reach the tissues of the intestinal wall through the blood stream. They rupture the blood vessel and cause a haemorrhagic nodule. Cysts were never seen in the small intestine, most of them are present in the submucosa, some are in the muscular layers and others are rarely found subperitoneally. From the photomicrographs the cysts described are mostly older nodules and would not, strictly speaking, conform to what can be regarded as young cysts which form when the larvae penetrate the mucous membrane in the first instance. He never found larvae in the walls of the intestine, but the adult stage only. Sometimes ruptured cysts become infected with bacteria from the intestinal flora and ulceration resulted. No worm nodules were found in other organs of the body. Those monkeys having large numbers of adult worms in the large intestine had diarrhoea. The author believes that substances secreted by the worms were not responsible for death. He believes death to be due to a septicaemia, although he could not cultivate any bacteria from the blood, but he states that the organisms probably did not grow on the media used. He found an adult female worm and a number of eggs in the morula stage in a nodule. In direct communication with this nodule was another, which possibly held a male worm which could have fertilised the female, before leaving its own nodule for the intestinal lumen.

Walferston Thomas (1910) describes what, according to him, is the second recorded case of oesophagostomiasis in the human subject, the first being described by Brumpt in 1905, quoted by him.

He has never seen a cyst above the level of the muscularis mucosae. This is interpreted to mean in the mucosa proper. He describes cysts in the submucosa and external muscular coat. The cyst consists of the worm and red cells in varying states of disintegration. Eosinophiles, neutrophiles and mono-nuclear cells are present in varying numbers in different cysts. Giant cells are also described, and in some cases there is calcification.

Hall (1920) in a popular article on parasites and parasitic diseases of sheep, mentions the presence of cysts in which the parasites may be present. These cysts contain necrotic material, yellowish or greenish in colour, and may be of a caseous or calcareous nature. Larvae which migrate to the mesenteric lymphatic glands, the omentum and the liver, probably die there and never get back to the intestine to complete their development. In severe cases there is emaciation and diarrhoea. He ascribes the bad effects produced by the worms to: (1) large portions of the intestinal mucous membrane are rendered functionless by the lesions produced therein by the parasites; (2) absorption of toxic substances from the worms themselves and from necrotic material from lesions produced by the parasites.

Theiler (1921) describes the post-mortem appearances of oesophagostomiasis in sheep as those of a pronounced anaemia, hydraemia and serous atrophy of fat. Nodules are present in the small and the large intestine. These he classifies arbitrarily as (1) the reddish nodule which is the young nodule; (2) the green nodule, which contains green pus and (3) the hard nodule in which calcification has taken place. Theiler further states that two serious complications may take place as a result of nodular worm infestation:—(1) Reksiekte* (Intussusception) and (2) septic infection of the serous cavities.

Joest (1926) describes the young nodule as consisting mainly of lymphocytes, together with the oesophagostomum larva in a relatively small amount of broken down tissue in which eosinophiles are present. Fibroblasts surround this central mass but a well defined connective tissue capsule has not yet formed. These young nodules are situated immediately under the muscularis mucosae. The larvae may migrate into the submucous tissues and the nodules may undergo caseation and necrosis. Sometimes ulcers develop.

Mönnig (1934) states that in lambs or in older sheep which have no resistance to the parasite the larvae cause practically no reaction, by their migration into the mucosa. In such cases a large number of adult worms can be found in the colon, without any nodules in the walls of the intestine. The fact that in some cases larvae pass into the submucosa, with the development of nodules, he ascribes to some degree of immunity which the mucosa appears to possess. The larvae according to this author may stay in the nodules for about three months when the contents become caseous and calcified, the parasites either die or leave the nodules to wander about between the muscle fibres. Some larvae may enter the blood and lymph vessels or pass into the abdominal cavity, producing nodules in the liver, lungs, myocardium and abdominal fat. The nodules are usually sterile, but larvae may carry bacteria into the walls of the intestine and produce acute inflammation, peritonitis or even abscesses. The worms are not blood suckers but feed on the intestinal contents or the predigested mucosa, after subjecting the mucosa to the action of secretions from the oesophageal glands. The worms secrete a toxic substance which has a harmful effect on the host.

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* An Afrikaans word, literally translated "stretching disease".

Wetzel (1934) believes that a glandular secretion from the nodular worms, causes a chronic inflammation of the intestinal mucous membrane of the sheep and that the parasites actually feed on the inflammatory exudate produced in this way.

LESIONS OF OESOPHAGOSTOMIASIS IN LAMBS.

Most of the material used in this study was the same as that used by Veglia (1923) when he was investigating the life history of this parasite. He infected lambs with large numbers of third stage larvae and killed these lambs at intervals of 12 hours, 24 hours, 36 hours, 2 days, 3 days, etc. Specimens of intestines were collected in formalin and these were kindly placed at my disposal by Veglia.

In addition to this material obtained from Veglia several other two-tooth merino sheep were experimentally infected with pure faeces cultures of Oesophagostomum larvae.

Serial sections of the intestine were cut and stained in the ordinary way with haemalum-eosin and van Giesen. In some cases the sections were specially stained with Giemsa, etc., for bacteria.

LESIONS IN THE INTESTINES OF YOUNG LAMBS EXPERIMENTALLY INFECTED WITH OESOPHAGOSTOMIASIS. (Veglia's material.)

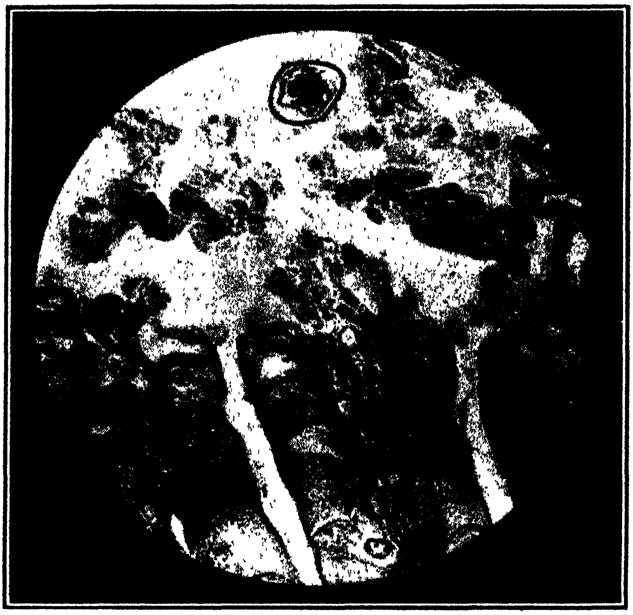
Control Lamb No. (2). *Specimen No.* 6569.

This lamb was not infected. The intestine had a normal appearance micro- and macroscopically. A careful examination was made for the presence of oesinophiles in the mucous membrane. Very few of these cells could be identified therein.

12 *Hours after infection.*

Lamb No. 31. *Specimen* 6570.—Small intestine—no parasites are seen either in or on the mucous membrane. Large intestine—no parasites are seen in the mucous membrane, but in some sections the parasites are seen lying on the mucous membrane. The larva in this case must be just on the point of entering the mucous membrane (see Plate I) or what is more likely is that a portion of the parasite has already entered the mucosa and that which is seen as apparently lying on the mucosa is merely a cross section of that portion of the larva which has not yet passed into the mucosa. If the larva is merely lying free on the mucous membrane it would probably not have remained there during the process of cutting and the manipulation of the sections during staining etc.

PLATE I.—Specimen 6570. 12 hours after infection



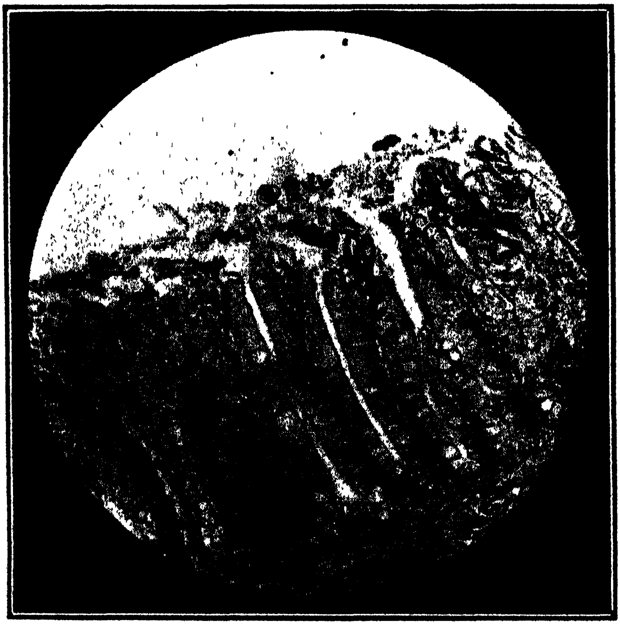


Fig. 1 (×150) and Fig. 2 (×450).—Larvae are just on the point of entering the mucous membrane of the large intestine.

As far as can be made out from a very careful microscopic examination of serial sections from the small and large intestine no evidence of any disturbances could be found.

24 *Hours after infection.*

Lamb No. 39. *Specimen* 6571.—Small intestine—Duodenum. There is very marked hyperaemia. The vessels are distended with blood. In some places the villi are markedly infiltrated with red cells. There is in addition a certain amount of desquamation of the cells of the mucous membrane. These lesions are possibly the early stages of a haemorrhagic enteritis (Plate II, Fig. 1 and 3), and probably directly or indirectly due to the effects of what can be conveniently described as primary parasitic migration, that is migration which is taking place when third stage larvae enter the mucous membrane and until they become encysted under the muscularis mucosae (i.e. against the muscularis mucosae and on its mucous membrane side). Many larvae are present. These are coiled up against the muscularis mucosae. This lesion has already appropriately been described by Veglia (1923) as a cyst. A very fine membrane forms the cyst wall which surrounds the larva. In addition to the larva one can recognise well preserved red cells in some cysts. (Plate III, Figs. 1 and 2) whilst in others there is a structureless (pink staining with eosin) fluid (Plate IV, Figs. 1 and 2). Evidence of inflammation or of bacteria was never seen within the cysts.

The question now arises as to how the cyst wall is formed. Goodey (1922) points out that the sheath of ensheathed larvae is produced by the old cuticle which is replaced by a new one developing underneath it. The possibility must therefore be considered that the old sheath of the larva actually forms the wall of the cyst. This would be the most economical means of supplying itself with this apparently protective covering. It has already been shown that the parasites are on the point of entering the mucous membrane 12 hours after infection and that many larvae are encysted 24 hours after infection. If a certain amount of time is allowed during which the parasite is making its way through the mucous membrane to become encysted under the muscularis mucosae, the cyst wall must in many cases be completed within 12 hours' time. It would be remarkable if the tissues would undertake this service so promptly on behalf of the parasite against its own interest. From the available histological evidence it would nevertheless seem, that this is actually what takes place. The cyst wall stains with 1 per cent. methyl-green and with haemalum eosin. With van Giesen it stains a light pink colour. Under oil emersion and with the light slightly cut off, the cyst wall has a granular appearance in places. Striation which is characteristic of the structure of the skin of, amongst others, nodular worm larvae, was never seen in the cyst wall, although it should be mentioned that in sections, one frequently fails to identify striation in the skin of encysted nodular worm larvae. On structural grounds one cannot differentiate the two on clear-cut lines. In some cysts there are numerous red cells between the coils of the larvae If the cyst wall is formed by the outer skin of the parasite, then these red cells must be between the two skins, which would seem to

PLATE II.—Specimen 6571. 24 hours after infection.

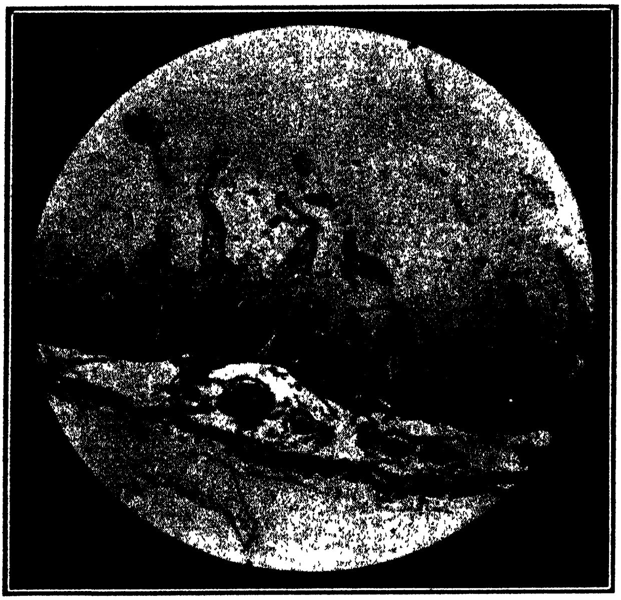


Fig. 1 (50×).—Duodenum haemorrhagic infiltration of mucous membrane.

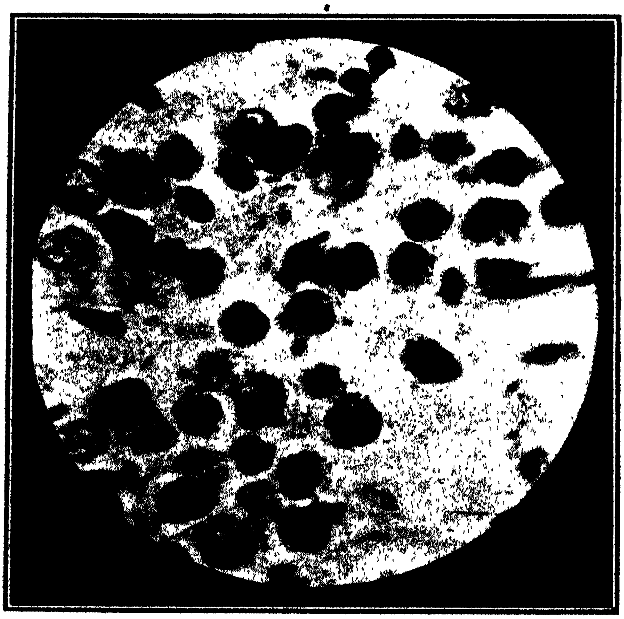


Fig. 2 (1200×).—Eosinophile infiltration of mucous membrane.

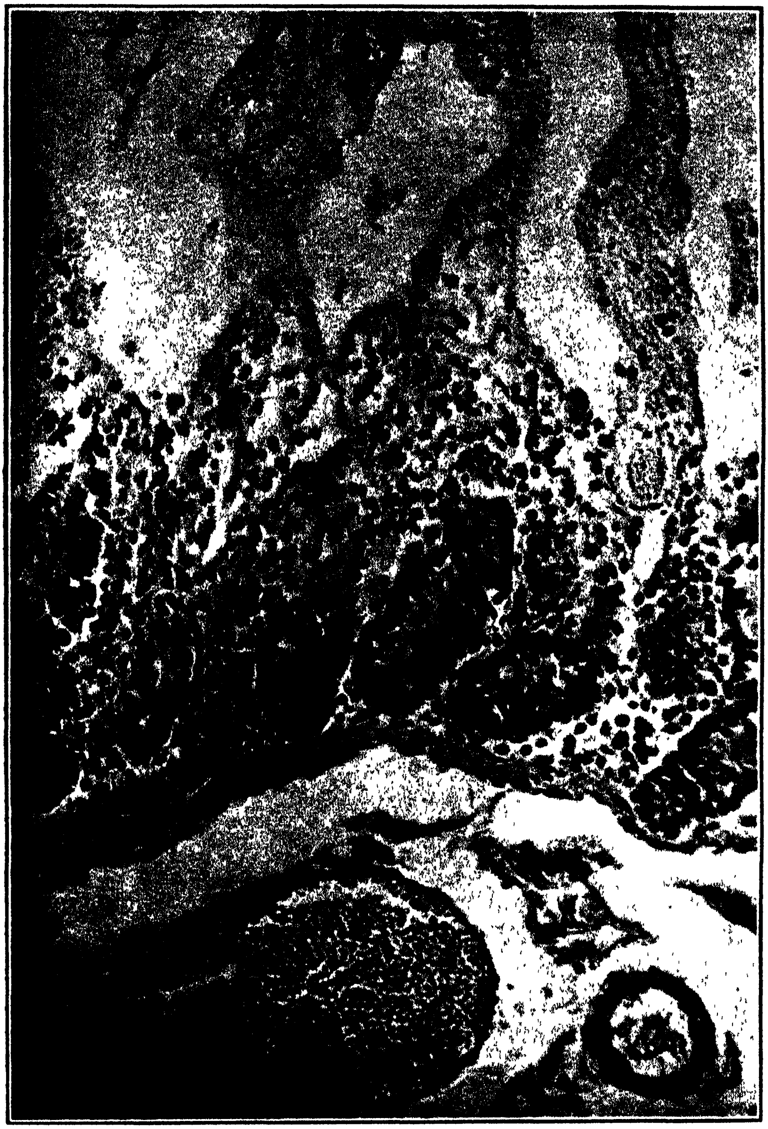


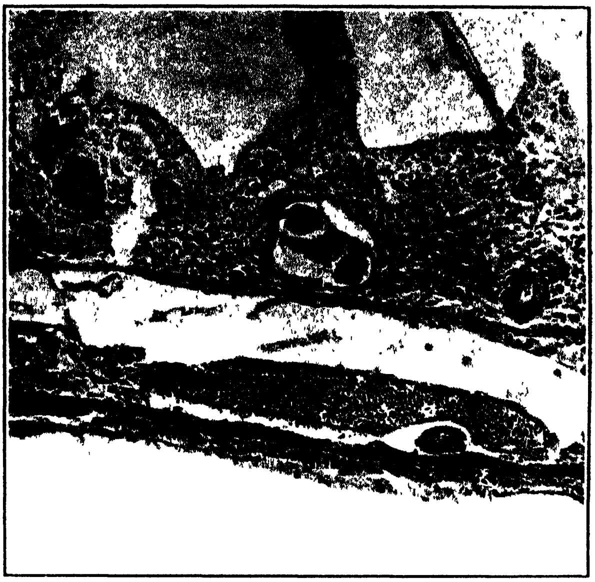
Fig. 3 (270×).—As Fig. 1, showing hyperaemia and haemorrhagic infiltration of villi.

be an exceedingly unlikely, if not an impossible eventuality. Even if the red cells should have been ingested by the parasite, they would not be present between the two skins, but in the alimentary canal of the parasite. Veglia (1923) further points out that the second ecdysis which occurs in the first parasitic stage of the socalled third stage larva is completed in the lumen of the alimentary canal of the sheep, very soon after the parasite is ingested. Thus having cast the old skin, it seems very unlikely that it could, within 24 hours, release the new skin to form a cyst wall. All the available evidence points to the formation of the cyst wall by or from the tissues.

Feng (1931) believes that in *Physaloptera clausa* infection of hedgehogs and *Physaloptera caucasica* infection of monkeys, the worms produce a secretion which causes liquefaction of the tissues. Spindler (1933) believes that such a liquefaction or more precisely a coagulative necrosis of the tissues may also be caused by the *Oesophagostomum longicaudum* in pigs and that this homogeneous tissue actually forms the cyst wall. As the parasite only remains encysted for a short time (4-5 days according to Veglia) it may be advantageous to have a protective covering which is not too strong. If such an envelope consists of dead tissue, this may automatically disintegrate in a few days time and release the parasite to complete its development. The great objection to this view is the fact that the cyst walls consist of such well-defined, albeit delicate thread-like structures, that it is hard to believe that a process of coagulative necrosis can produce them. Histological evidence which may to some extent support Spindler's contention is furnished by a cyst which is reproduced in Figs. 1 and 2, Plate VI. From the walls of this cyst one sees in places what appear to be hooklike processes projecting into the cyst cavity. If there was a mass of liquefied tissue which the parasite pushed out in the form of a cyst wall, one can well imagine that some of this substance could flow or be forced between the coils of the larva and so produce these hooklike processes.

However on careful examination with the higher magnifications one can differentiate what can be described as the membranous cyst wall, as a well-defined delicate thread running round the hooklike process. In addition the two stain differently with van Giesen, and the structureless material inside the cyst cavity can similarly be differentially stained. However too great importance should not be attached to this, as the material inside the cysts may be present as a thin layer, as a result of which it may stain more lightly than the thicker cyst wall. The exact nature of the structureless material which is present in some cysts (Plate IV, Figs. 1 and 2) has not been determined. If it is not the result of a process of liquefaction, is it secreted by the parasite, or by the wall of the cyst? Some cysts as already clearly shown undoubtedly contain red cells and it seems very likely that the structureless material is the remains of these red cells after they had become haemolysed. In view of the foregoing the question of the formation of the cyst wall must for the time being remain open. In the case of one cyst (5 days after infection, Fig. 3, Plate IV) a nucleus resembling that of a fibroblast seems to be present in the cyst wall and the possibility that it may consist of some form of connective tissue cannot be entirely excluded. It is rather remarkable that in all cases the larvae become encysted immediately against the muscularis mucosae and that encysted larvae are never seen in the wall of the intestine deeper than the muscularis mucosae. It seems very likely that this structure merely offers a convenient mechanical barrier to the progress of the larvae and as there is no particular reason why they should go further, they become encysted there.

PLATE III.—Specimen 6571. 24 hours after infection.



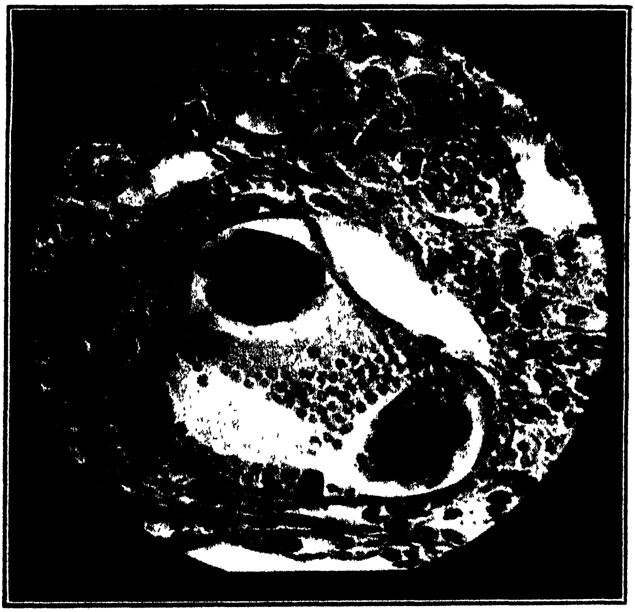


Fig. 1 (×150) and Fig. 2 (×450).—Duodenum. Larva encysted under muscularis mucosae.

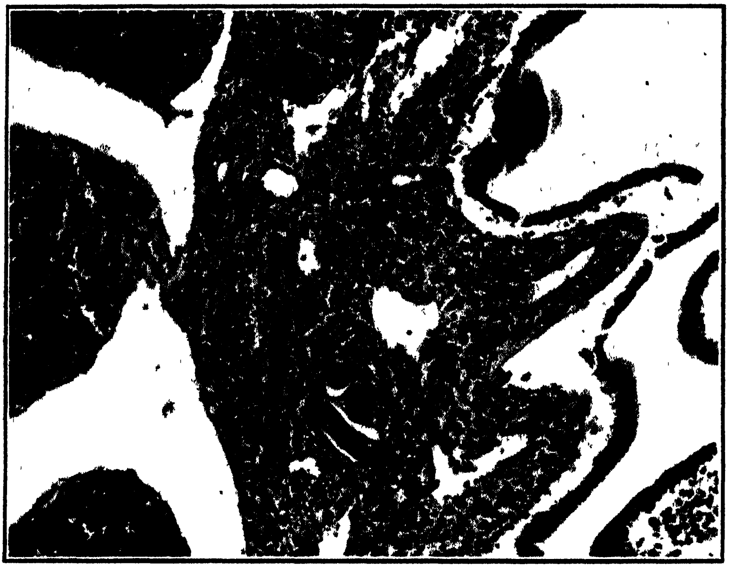
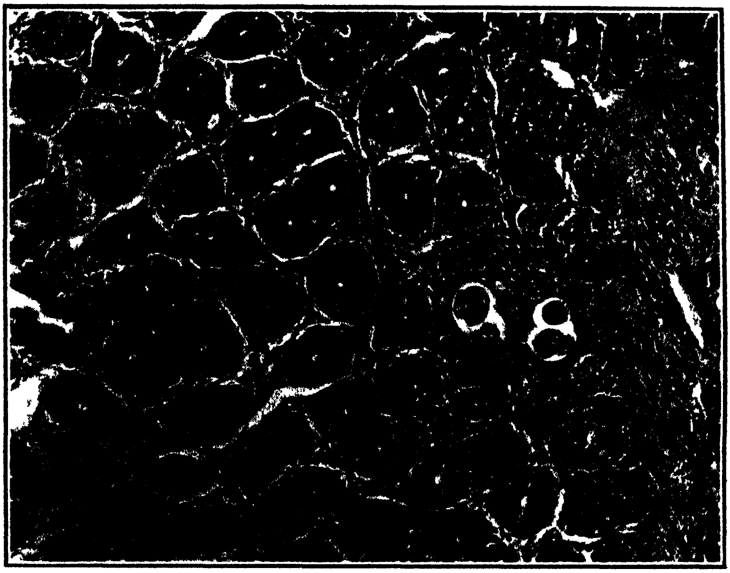


Fig. 3 (150×).—Encysted larva under muscularis mucosa—terminal portion Ileum.

Already at this early stage abnormal numbers of eosinophiles are present in the mucous membrane. Plate II, Fig. 2. Many of these cells seem to have round nuclei, but one hesitates in making a definite statement to this effect, as the cells may be lying in such a way that one may not be able to see the other lobes of the nuclei. If one is actually dealing here with eosinophile myelocytes, they must have developed locally from lymphoblasts (hemocytoblasts of the unitarians). Maximow 1923(b) quoted by Maximow (1928) showed that lymphocytes may even in tissue culture be induced to differentiate into amongst others eosinophile myelocytes. It seems very unlikely that the bone marrow could react so quickly that these cells could function in the intestine within a period of 24 hours. If it is merely a question of the mobilization of the eosinophiles, one can understand their rapid withdrawal from the blood, but then one could not be dealing with myelocytes. These cells occur free and not in epithelial cells and were therefore not confused with the "Schollenleukozyt" described by Weill, quoted by Keasbey (1923).

These eosinophiles are diffusely present almost throughout the mucous membrane but seem to be more numerous in the neighbourhood of larvae. Seeing that the animal was exposed to a gross infection one can expect that parasites are entering the mucous membrane almost along the entire line of the intestine, this probably explains their diffuse distribution. In the control uninfected lamb, hardly any eosinophiles are present. Similar lesions are present in the Ileum, (Plate III, Fig. 3). Here also numerous eosinophiles are seen to be diffusely present throughout the mucous membrane. This portion of the intestine does not contain lesions like those of the early stages of a haemorrhagic enteritis to the same extent as the duodenum.

PLATE IV.—Specimen 6571 (Figs. 1 and 2). 24 hours after infection.



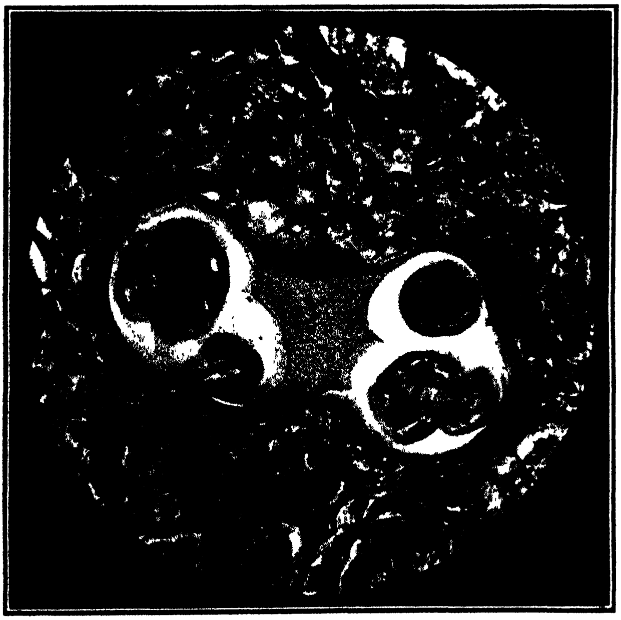


Fig. 1 (150×) and Fig. 2 (450×).—Large intestine. Larva encysted under muscularis mucosae.

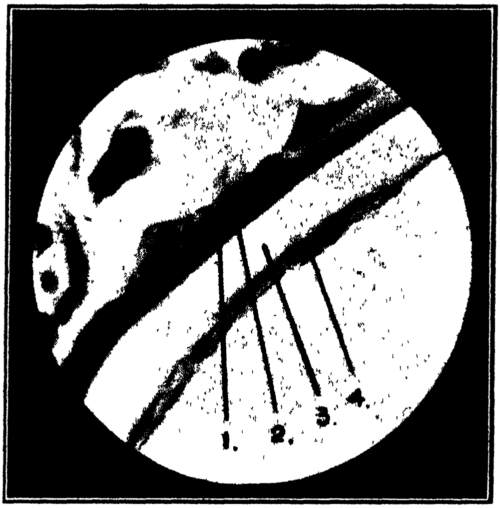


Fig. 3, Spec. 6578. 6 days after infection. (1500×).—1 nucleus fibroblast, 2 cyst wall, 3 cyst cavity, 4 portion of encysted larva.

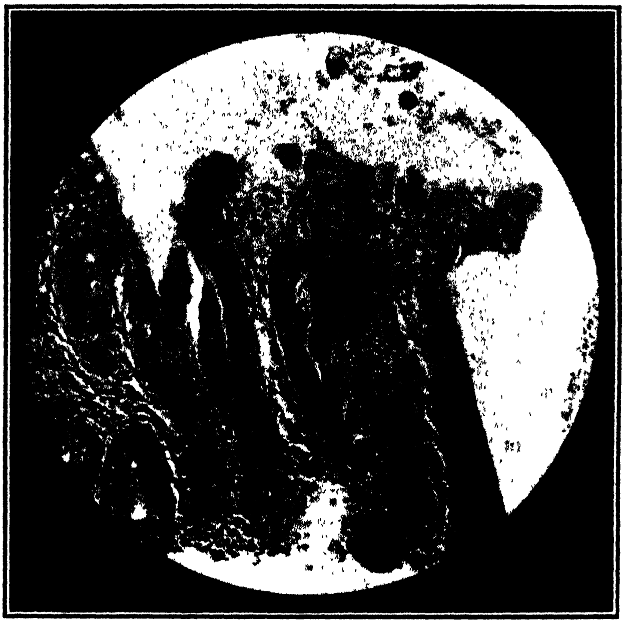
Large Intestine.—As the encysted larvae lie coiled up against the muscularis mucosae, they are cut in several places in cross section. This is clearly seen in Plate IV, Figs. 1 and 2. Immediately around the cyst wall there are quite numerous eosinophiles. This would seem to be quite remarkable if it is remembered that the larvae had probably only ventured into the mucous membrane during the previous 12 hours. In Plate V a larva is seen more or less in longitudinal section. It is not possible to make out definitely which is the cranial portion of the parasite. However, it would seem that the larva is entering the mucous membrane between two glands. The presence of a considerable number of eosinophiles is very definite evidence that the tissues are resisting this invasion; not only immediately around the parasite are numerous eosinophiles, but these cells are also prominently present in the tissues some distance away from the parasites. Similar lesions are present in the small and large intestine of another lamb No. 36 destroyed 24 hours after infection.

48 *Hours after Infection.*

Specimens from three different lambs were available for examination.

No. (1). *Lamb No.* 129. *Specimen* 6572.—Encysted larvae are present under the muscularis mucosae, whilst others are still migrating apparently on their way to the muscularis mucosae. In this particular lamb the eosinophile reaction, although present, is not so well marked as in the previous case.

PLATE V.—Specimen 6571 (Figs. 1 and 2). 24 hours after infection. Specimen 6574 (Fig. 3). 3 days after infection.



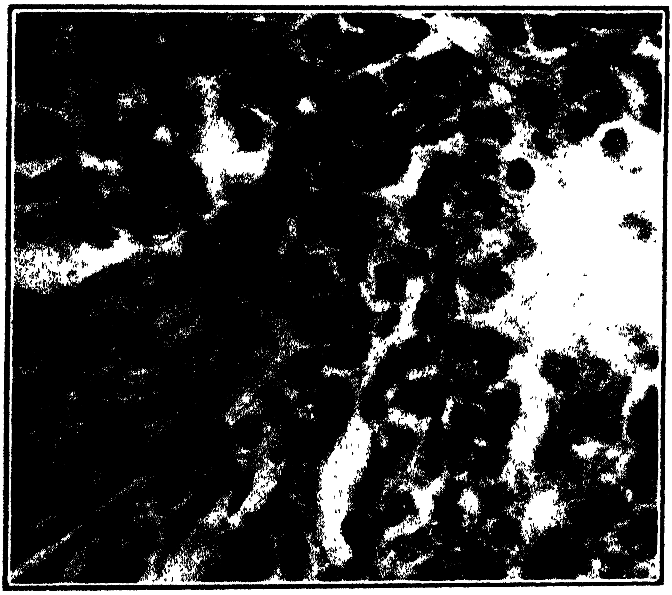


Fig. 1, 6571 (150×) and Fig 2, 6571 (850×).—Larva entering mucous membrane between two glands. Eosinophile infiltration around parasite.

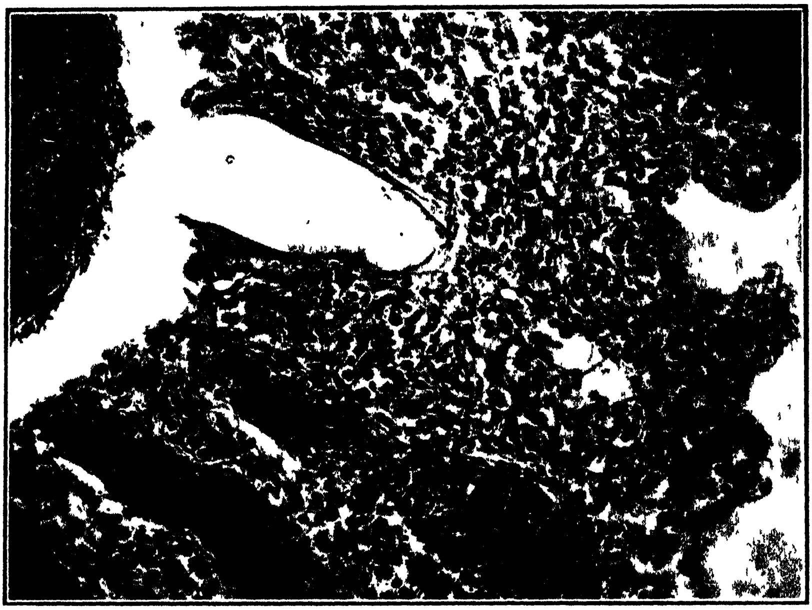


Fig. 3, Spec. 6574 (250×). Empty cyst 3 days after infection

No. (2). *Specimen* 6912—*Additional Lamb.*—Whilst no parasites are seen in sections examined from the jejunum, ileum, caecum and ansa spiralis, parasites are found on the mucous membrane apparently on the point of entering the duodenum, the junction of the caecum and ansa proximalis and the rectum. Eosinophiles in appreciable numbers are present throughout the mucous membrane of the small and large intestine. This eosinophile reaction is probably due to the presence of larvae which had already entered the mucous membrane on their primary parasitic migration, although no such parasites could be demonstrated in the depth of the mucous membrane and no encysted larvae were found in hundreds of sections examined. The method of infection will probably influence to a considerable extent the rapidity with which the parasites pass through their various developmental stages. If the larvae must first of all enter the rumen, it will take them longer to become encysted than if they should pass through into the abomasum and this may possibly explain the apparently delayed development in this lamb.

No. (3). *Lamb No.* 38. *Specimen* 6573.—No parasites were found in any of the serial sections examined. With the exception of a catarrhal enteritis in the ileum no lesions were recognised in the small or large intestine.

3 Days after Infection.

Lamb No. 18. *Specimen No.* 6574.—Encysted larvae are present against the muscularis mucosae of the large intestine. In the vicinity of the cyst are scattered eosinophiles. In some places empty cysts are seen (Plate V, Fig. 3). The ruptured end of the cyst is immediately opposite the muscularis mucosae. On following a sort of track which is present between the mucous membrane and the muscularis mucosae, portions of a migrating larva, which could possibly have migrated from this cyst, are found. The larva was unfortunately cut in such a way that it could not be definitely identified as a fourth stage larva. From this it would appear that what will be described as secondary parasitic migration in this paper, can sometimes take place considerably earlier than 5 days, which according to Veglia (1923) is the time when the fourth stage larvae usually emerge from their cysts.

4 *Days after Infection.*

Lamb No. 36. Specimen No. 6575.—No parasites were found in the sections cut from the first portion of the small intestine. In the ileum numerous encysted larvae are present under the muscularis mucosae. The cysts are now increased in size and can readily be picked out under an ordinary dissecting microscope with a magnification of 16. The larvae can only grow if they have sufficient available food material. It seems unlikely that they can build up sufficient reserve material during the short time of their primary parasitic migration, for their growth while they are encysted. If such reserve materials are not available the larvae probably make use of red cells and possibly other substances present within the cysts for food. In some of the sections photomicrographs of which are reproduced (Plate VI) the cyst wall is clearly seen to consist of a

PLATE VI.—Specimen 6575. 4 days after infection.

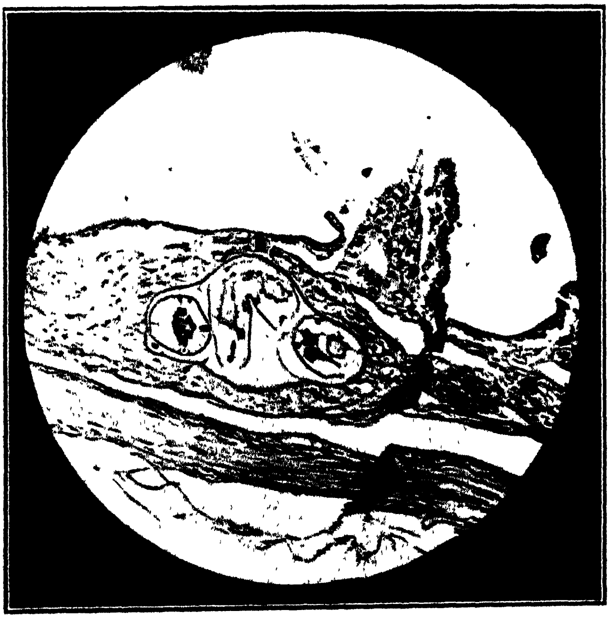


Fig. 1 (150×).—Encysted larva. Ileum.

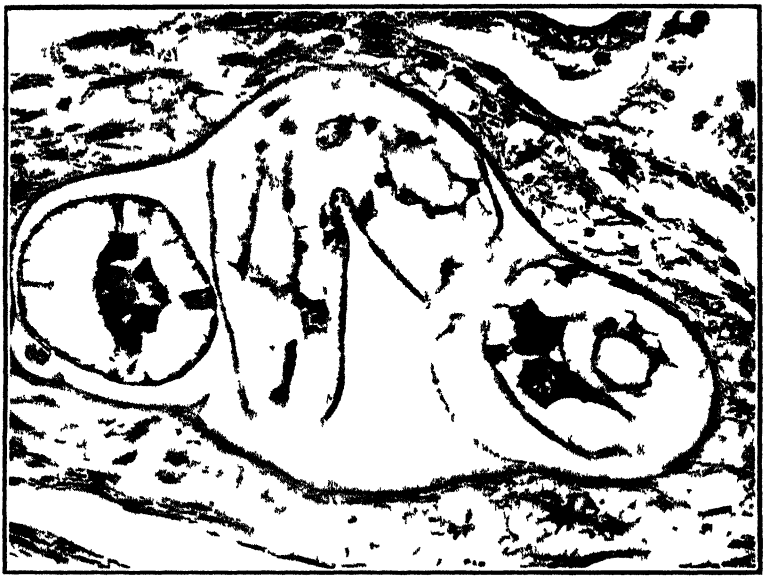


Fig 2 (450×) —As Fig 1

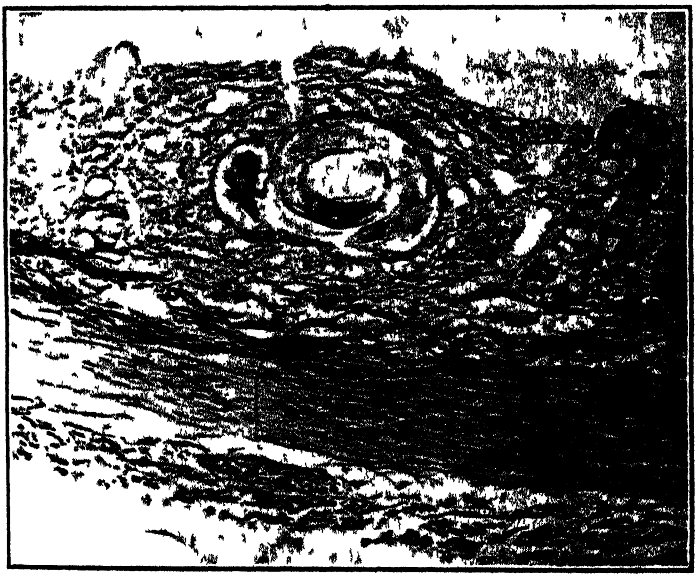


Fig 3 (150×) —Encysted larva Large intestine fine thread like filament, which at this stage can be differentiated from the outer skin of the parasite by van Giesen's staining. The outer skin of the parasite takes on a yellowish colour whereas the cyst wall stains a light pink colour. The glandular epithelium of the large intestine is in places completely replaced by the cyst and by a zone of tissue which has formed around the cyst. This zone of tissue consists of scattered eosinophiles, fair numbers of round cells, but mainly of cells having large vesicular or spindle shaped nuclei, which are regarded as fibroblasts, amongst which a certain number of epithelioid cells may also be present. This probably represents a type of granulation tissue, which already at this early stage seeks to encapsulate the parasite. This cellular zone around the cyst varies in depth, as it consists of only a few rows of cells in the case of some cysts, whilst in the case of others an appreciable zone of tissue is formed around the cyst.

Numerous serial sections were examined from various portions of the gut and in not a single one was any evidence found that larvae had penetrated the muscularis mucosae.

5 *Days after Infection.*

Lamb (*no number*). *Specimen* 6577.—Many encysted larvae are present against the muscularis mucosae. In Fig. 1, Plate VII two larvae are shown encysted side by side. If the tissues are responsible for the cyst wall, it seems strange that they did not include these two larvae with their associated cyst contents within one cyst wall, there is, however, a possibility that the two larvae became encysted at different times. The cyst generally resembles that which is seen four days after infection. In serial sections from another portion of the large intestine a portion of a larva is seen more or less in longitudinal section, Fig. 2 Plate VII. The head end is against the muscularis mucosae and causes a bulging of it towards the submucosa. Judging from the mouth capsule etc. the structure of this larva conforms to the structure of the fourth stage larva described by Veglia (1923). According to Veglia, Oesophagostomum larvae are, during this time, completing the third ecdysis but they had not yet migrated back to the lumen of the intestine. This larva must have emerged from the cyst and is now migrating as the fourth stage larva. This can be conveniently described as secondary parasitic migration, in contradistinction to what has already been referred to as primary parasitic migration.

6 *Days after Infection.*

Lamb No. 16074 (*Own case*). *Specimen No.* 6578.—Numerous encysted larvae are still present against the muscularis mucosae (Plate VIII). The cysts themselves vary a good deal in appearance. Some have a well-defined delicate threadlike membranous wall (Fig. 4) with very few cells ordinarily associated with granulation tissue and capsule formation, whereas others have in addition a zone of tissue consisting of several rows of cells (Fig. 3). The cells of the central rows, i.e. immediately around the parasite have large vesicular nuclei, some of which are almost elliptical in shape, others more or less round. The cells of the peripheral rows have nuclei

PLATE VII.—Specimen 6577. 5 days after infection.

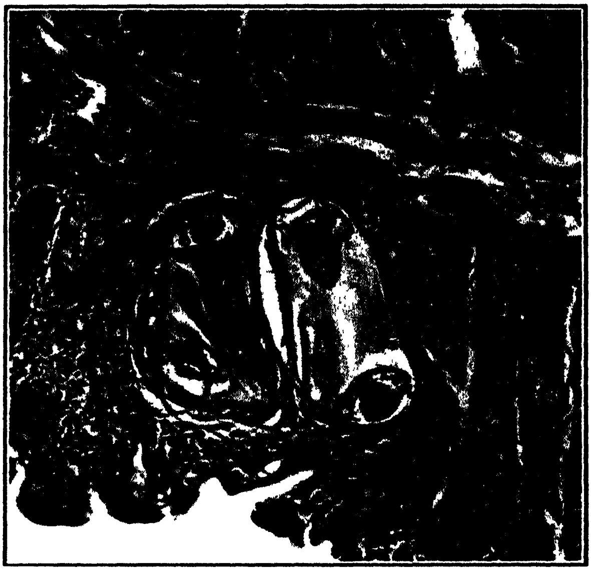


Fig. 1 (150×).—Encysted larvae. Large intestine.

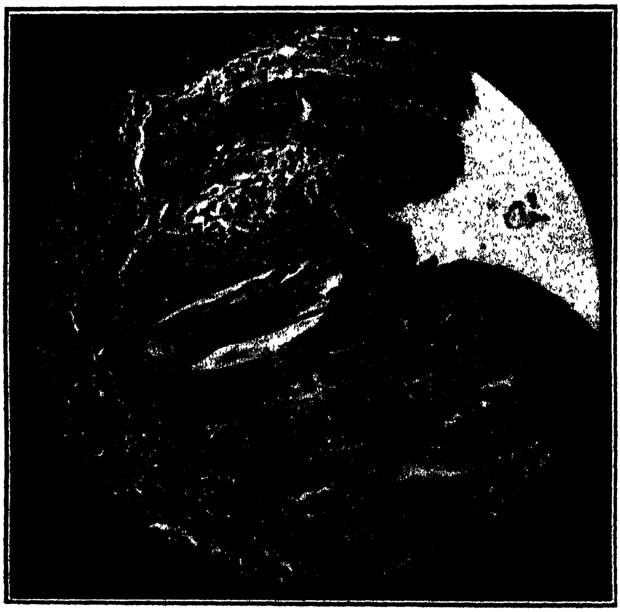


Fig. 2 (150×).—Migrating 4th Stage larva.

which are spindle shaped. These cells are interpreted as being mainly fibroblasts and the tissue is regarded as granulation tissue, which is building a capsule around the parasite. This difference in the structure of the cysts may be due to: (1) a difference in age. It is almost certain that even in cases where infection takes place at the same moment, some larvae will complete their development before others. (2) For some unknown reason, the tissue reactions to individual cysts may vary. It seems unlikely that there would be an inherent difference in the cysts themselves, but possibly the kind of tissue where they become encysted, may influence the type of reaction which is produced.

In places there are empty spaces from which the larvae have migrated. In addition to migrating larvae of the fourth larva or second parasitic stage, the nodules which are produced by some of them are now for the first time seen. (Plates VIII and IX). The nodules are present mainly in the submucous tissues, but some are present in the submucous as well as the mucous tissues. In the earlier sections of the series, one sees merely a more or less circumscribed accumulation of eosinophiles, but in later sections of the series portions of the larvae are easily recognised. In Fig. 1 Plate IX the nodule is seen to occupy the entire mucous membrane and penetrates the muscularis mucosae to extend into the submucous tissues. Here the larva is seen cut more or less longitudinally and details in its structure are easily recognised. The head end lies in the submucosa and is directed away from the mucous membrane. The entire parasite is closely surrounded by infiltrating cells amongst

Plate VIII.—Specimen 6578. 6 days after infection.

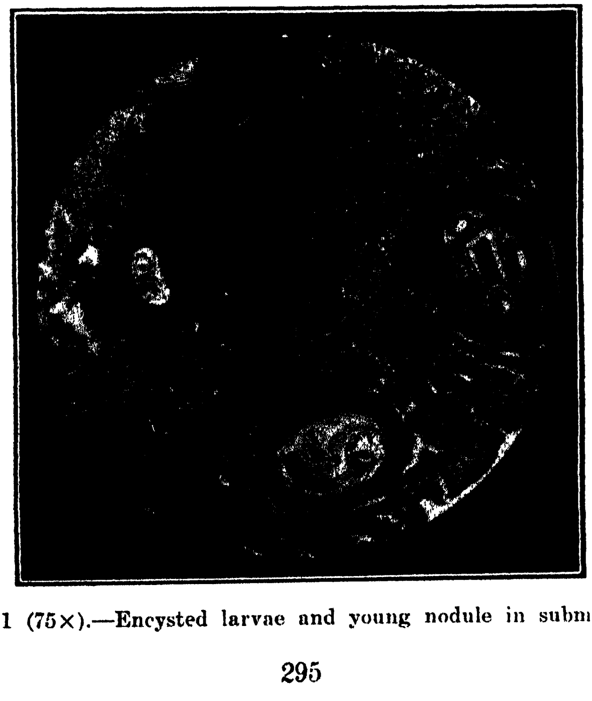


Fig. 1 (75×).—Encysted larvae and young nodule in submucosa.

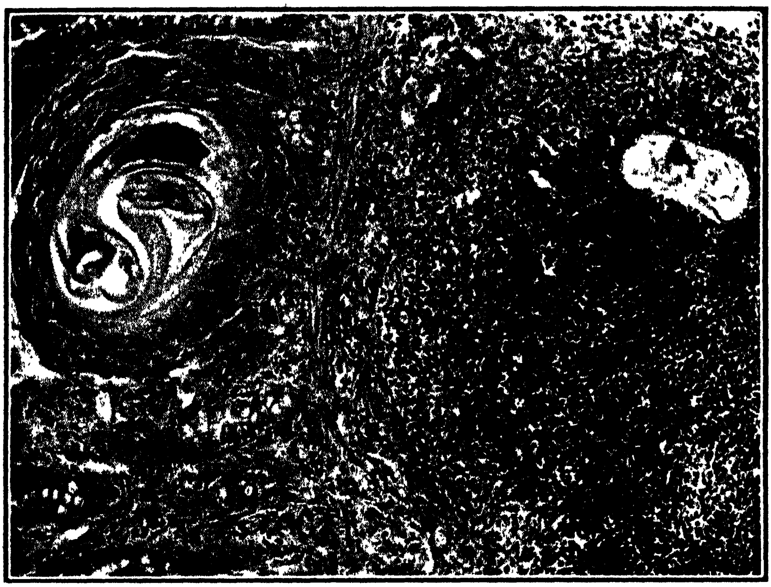


Fig. 2 (150×).—As in Fig. 1.

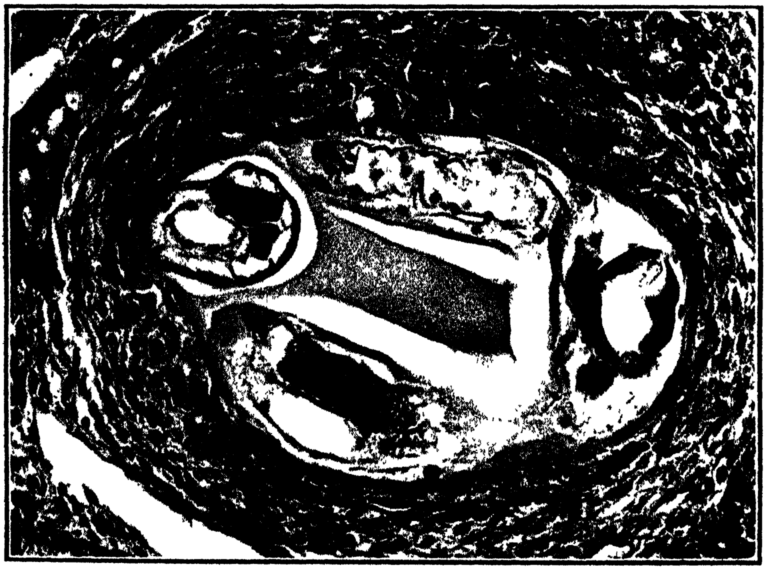


Fig. 3 (300×).—As in Fig. 2. Well developed capsule.

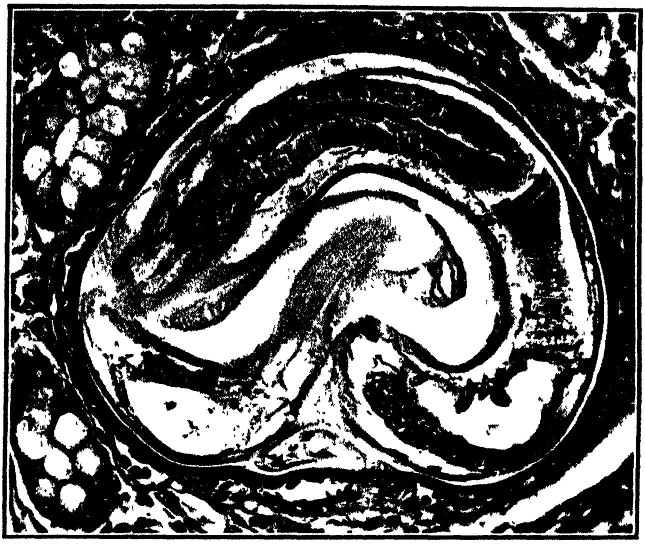


Fig. 4 (300×).—No capsule formation.

PLATE IX. Specimen 6578. 6 days after infection.

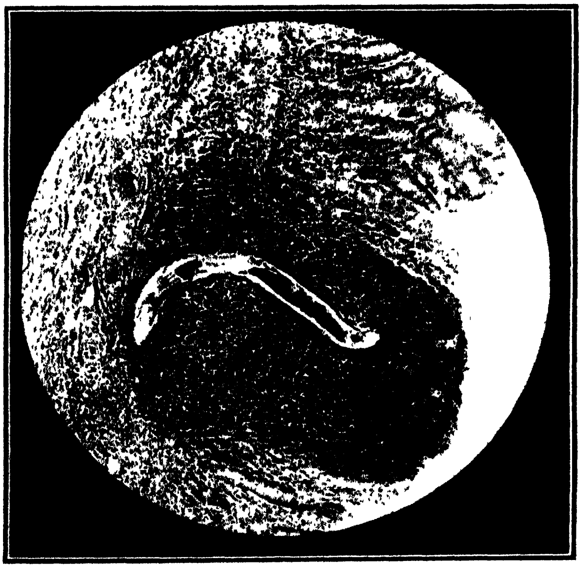


Fig. 1 (75×).—Worm nodule in mucosa and submucosa.

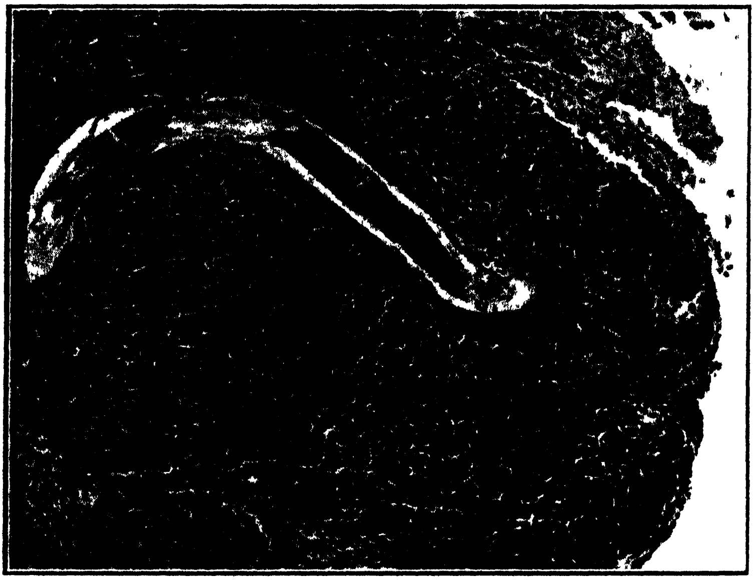


Fig. 2 (150×).—As Fig. 1.

which many eosinophiles are present. In other sections where a larva is cut mainly through its cranial portion, a group of polymorpho-nuclear cells is seen lying at the entrance to the oesophagus. Only the nuclei of these cells can be identified. They are regarded as eosinophiles which the larva had ingested, as there is no reason to suppose that these cells could have been accidentally deposited here in such a regular group during the process of cutting. The early nodules as seen in these sections consist of the parasite with masses of infiltrating cells, the great majority of which are eosinophiles. In addition to such circumscribed accumulations of eosinophiles around the migrating larvae, there is also a diffuse infiltration of eosinophiles throughout the mucous membrane. This must be looked upon as an expression of the defensive mechanism of the intestine, which is being mobilized against the parasites.

7 Days after Infection.

Lamb No. 19. *Specimen* 6579.—In quite a number of places there are bloody tracts in the mucous membrane. These may extend to the surface of the mucous membrane, where the parasite has passed out into the lumen of the gut. Migrating and encysted larvae are present in the mucous and submucous tissues of the large intestine. In the case of the ileum larvae are seen wandering in the interstitial tissues of the follicles in Peyers patches, Plate X, Fig. 1.

PLATE X.—Specimen 6579. 7 days after infection.

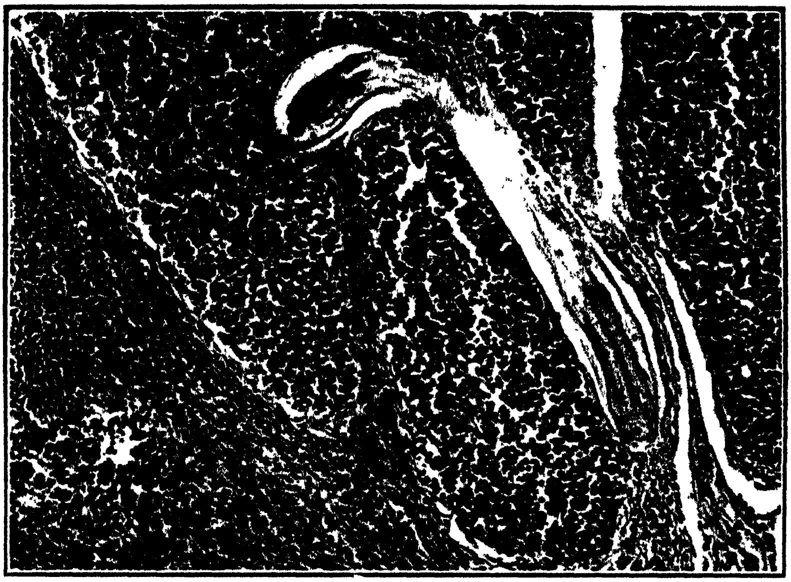


Fig. 1 (150×). Migrating larva in follicle of Peyers Patch.

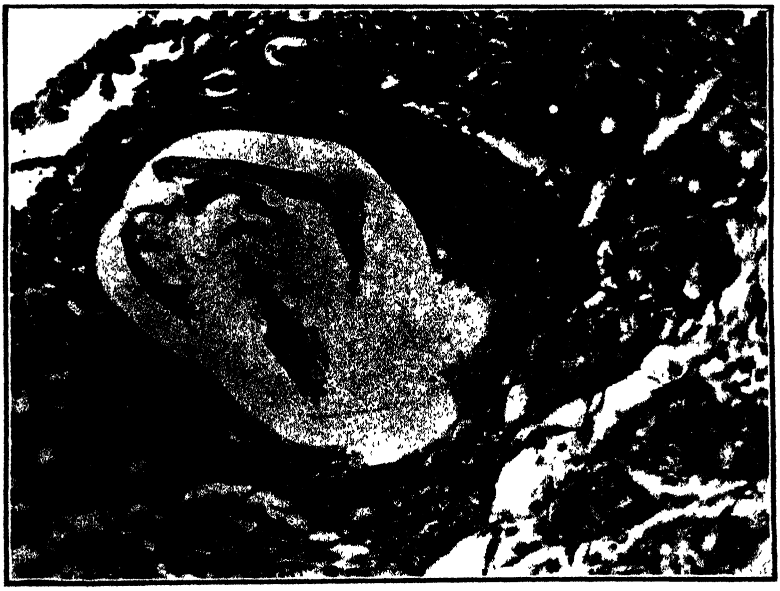


Fig. 2 (225×).—Empty cyst.

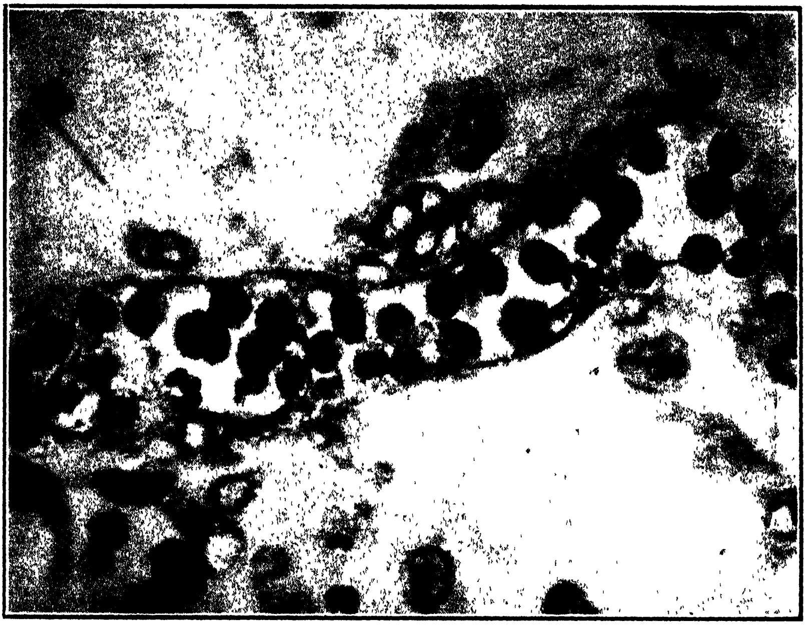


Fig. 3 (1000×).—Blood vessel in submucosa containing numerous eosinophiles.

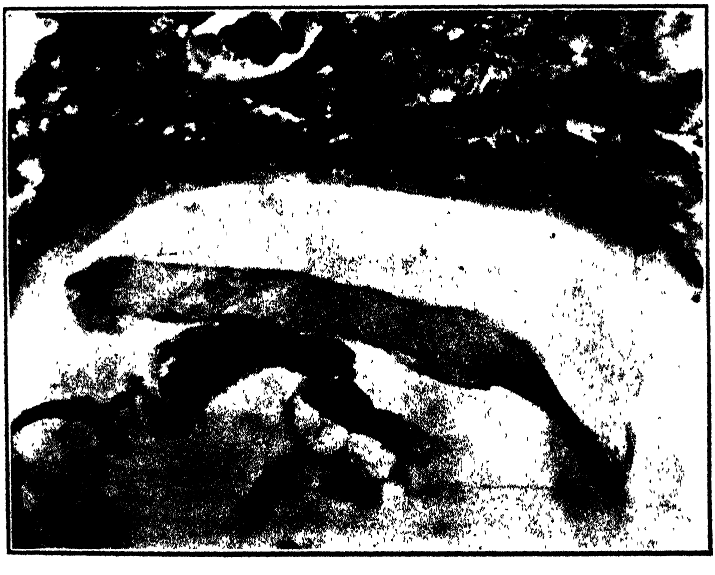


Fig. 4 (650×).—Showing cast striated skin.

The migrating larvae especially in the submucous tissues are closely surrounded by infiltrating eosinophiles, forming typical young worm nodules as in the previous case six days after infection. Eosinophiles are also diffusely distributed in the mucous membrane. Although the available evidence is inconclusive there would seem to be a possibility that adult worms and to a less extent fourth stage larvae, in the lumen of the intestine, may induce a diffuse eosinophilia in the mucous membrane. Further, eosinophiles are seen to be the dominating cells in certain vessels in the submucosa (Fig. 3, Plate X). These are undoubtedly blood vessels as they contain red cells in addition to the eosinophiles. They are not capillaries, as they are far too wide and can easily accommodate 3 or 4 eosinophiles side by side. Neither are they arterioles as their walls are much too thin. Furthermore, if they were arterioles one must assume that there is active haemopoiesis in the myeloid tissues, from which the eosinophiles were released into the circulating blood. Judging from the preponderance of eosinophiles in these vessels, the vast majority of the leucocytes in the blood ought in that case to be eosinophiles, a condition not yet encountered to such a marked degree in the fairly extensive haematological observations made in cases of oesophagostomiasis. One is therefore forced to the conclusion that these vessels are actually venules, which are draining an area where active secondary migrating larvae are present or from which such larvae are disappearing. When the eosinophiles are passed into the general circulation their numbers will relatively be very much decreased, but they may still be sufficient to produce a transitory eosinophilia. Later, however, they will disappear from the circulation. If this interpretation is correct, an eosinophilia in oesophagostomiasis is not so much an indication of an active tissue verminosis, but rather, that the tissue verminosis has been completed. In such cases one visualizes migrating larvae which penetrate the muscularis mucosae, wander about in the submucous and other tissues, where an intense eosinophilic reaction is elicited. Should they die and find their way back into the lumen of the gut, the eosinophiles may no longer be required for the purposes of defence and are released into the general circulation, from which they will later disappear. Hadwen (1925) had already at that time expressed the view that this may occur in cases of parasitism.

In some sections empty cysts are seen (Fig. 2 and 4. Plate X). The walls of the cysts are intact except at the one end where the parasite has broken through. The suggestion previously put viz. that the walls which were supposed to consist of dead tissue, will disintegrate to release the parasite, is not supported by the structure of this empty cyst, which does not differ from that of the inhabited cyst. When the parasite has reached the end of the first parasitic stage it casts its skin, the striated structure of which is easily recognised (Plate X, Fig. 4). The parasite then breaks through at one end; leaving, as already stated, the remaining portion of the cyst wall intact. The cyst wall, as a definite structural entity, is very clearly seen in the empty cyst reproduced in Plate XI, Fig. 1.

8 *Days after Infection.*

Lamb No. 3585. *Specimen* 6580.—Encysted larvae are only very rarely seen against the muscularis mucosae, but migrating larvae and nodules produced by them in the walls of the intestines are frequently seen. The nodules are present mainly in the submucous tissues, but in places portions of the mucous membrane itself are etxensively involved, with the complete destruction of a considerable number of villi. In other cases the muscular layers are extensively replaced by eosinophiles. Some of the infiltrating cells have undergone necrosis but in places one can still recognise the polymorphic nature of nuclei of what are probably the remains mainly of broken down eosinophiles. Round cells are also present. This probably represents the early stages of caseation (Figs. 2 and 3, Plate XI), although it should be mentioned that in some of these centres showing commencing necrosis, quite a number of bacteria are present. They are bacilli, all apparently of the same type (Plate XI, Figs. 2 and 3). They are probably not putrefactive bacteria, as there are portions of the same section in which they are not present. (Decomposition can be definitely excluded as the lamb was destroyed and specimens collected in formalin almost immediately after death). It seems as it the migrating larvae have produced such severe changes in the mucous membrane that bacterial infection from the lumen of the bowel took place and one may then be actually dealing with a true inflammatory process in addition to the lesions produced by the worms themselves. The fact that neutrophiles are undoubtedly also present further supports this. However, to anticipate what will be shown later in this paper, it does not seem that bacterial infection is a regular complication of oesophagostomiasis, but seems especially liable to occur when migrating larvae produce extensive lesions in the mucous membrane. Occasionally empty cysts are seen under the muscularis mucosae (Plate XI, Fig. 1).

PLATE XI.—Specimen 6580. 8 days after infection

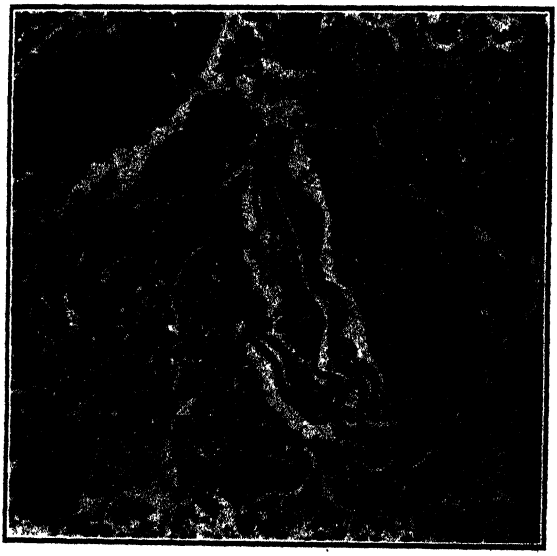


Fig. 1 (250×).—Empty cyst showing structural entity of cyst wall.

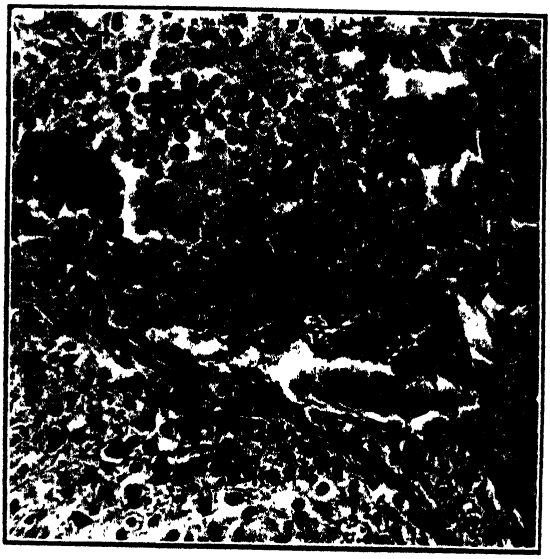


Fig 2 (375×).—Commencing necrosis with bacteria.

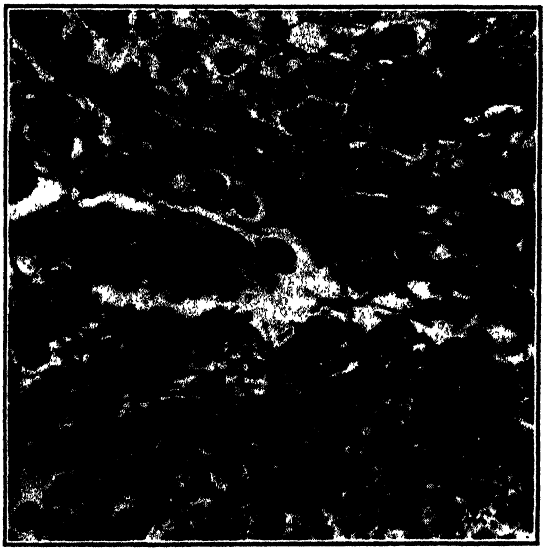


Fig. 3 (750×).—As Fig. 2.

12 *Days after Infection.*
(Two lambs.)

Lamb No. 1. *Specimen* 6581. *Large Intestine.*—Nodules of varying size are present in the mucous membrane, the submucous tissues, the longitudinal and circular muscular layers, and in some cases extending right up to the serosa, which seems to be on the point of being perforated. In these situations the normal tissue has been completely destroyed (Plate XII) and replaced by the nodules. In some places a nodule may be seen to extend from the muscular layers, through the submucosa, right to the surface of the mucous membrane, forming an ulcer which opens into the lumen of the gut.

The nodule at this stage has the following structure. In a few cases the cells (eosinophiles) which mainly form the nodules, are still seen more or less intact, but most of the nodules consist of a large central structureless mass, which stains more or less uniformly pink with eosin. In nodules from specimens which are cut and stained, soon after they are collected, the central structureless mass stains an intense pink colour with eosin, differing in this respect very markedly from the glanders nodule, the Preisz-Nocard abscess and the caseating tubercle. In places nuclear debris can still be seen and this is always in greater abundance towards the periphery of the nodule, where a well defined more deeply staining zone in which the nuclei of the cells are seen in varying stages of necrosis, is usually present. Here intact eosinophiles may be seen and where the living and dead tissues meet the cells of the tissue normal to the part are pycnotic.

PLATE XII.—Specimen 6581. 12 days after infection.

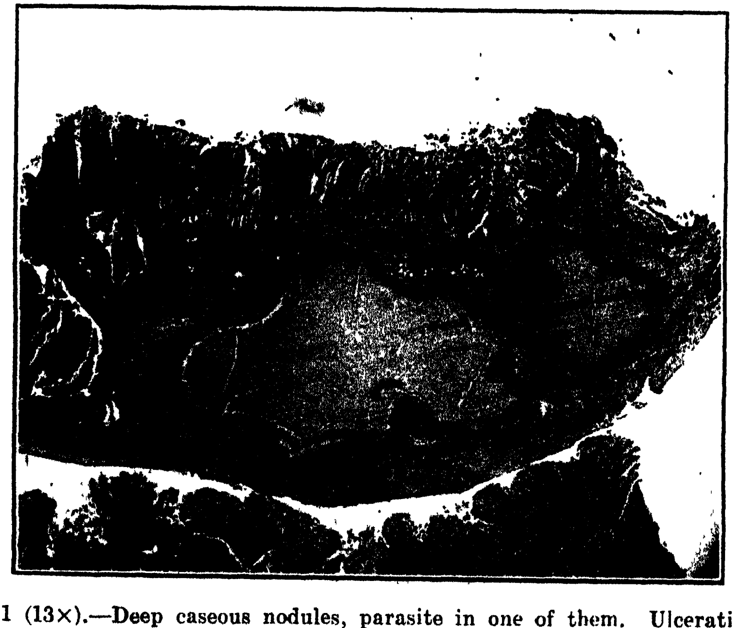


Fig. 1 (13×).—Deep caseous nodules, parasite in one of them. Ulceration of mucous membrane.

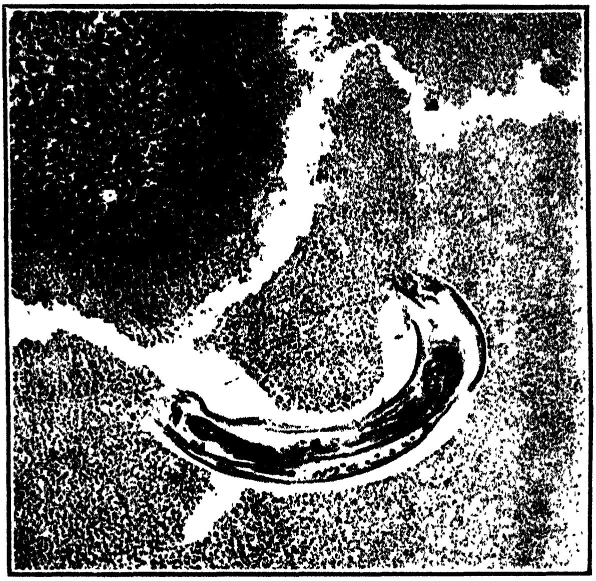


Fig. 2 (150×).—As in Fig. 1.

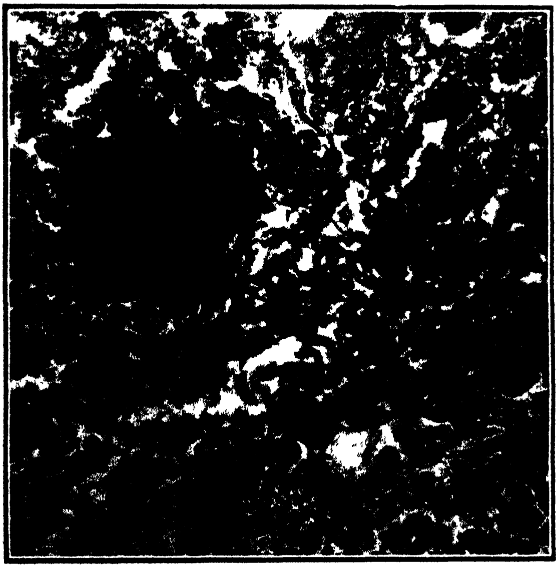


Fig. 3 (750×).—Another nodule, commencing necrosis, numerous bacteria.

In addition to the remains of broken down leucocytes, mainly eosinophiles, but in some cases probably also neutrophiles, one may find in the central caseating mass the following:—

(1) *Bacteria.*—These are present in most of the nodules. They may be scattered throughout the central structureless mass or may occur in groups towards the periphery of the nodule. These bacilli seem to be exactly like those previously described. They appear to be Gram positive rods, and here and there small bodies, resembling spores are present. These bacteria are present not only in the depths of ulcerating lesions, but also in nodules deeply situated in the submucosa, including the muscular layers of the intestinal wall. These bacteria probably gained entrance through the severe lesions produced by the gross parasitic infestation. It is extremely unlikely that the parasites carried them into the walls of the intestine, when they first entered the mucous membrane. If that were so, one would expect to find evidence of inflammation (regenerative or degenerative) in the walls of the intestine, when the parasitic invasion first takes place and when the cysts are formed. This is, however, not the case, and in the cysts themselves one never sees any bacteria or any inflammatory reaction. This is confirmatory evidence in support of the views of Carne and Clunies Ross (1932) who failed to find any Preisz-Nocard organisms in the nodular worm lesions of sheep, which were dosed *per os* with nodular worm larvae as well as with cultures of the Preisz-Nocard organism. Further it is of interest to note that these authors in examining, histologically and culturally, 50 well-developed lesions of oesophagostomiasis of the bowel wall of sheep, were able to identify gram-negative bacilli of the colon-typhoid type in two instances. If infection by paratyphoid bacilli was more common in sheep, one would have to consider the possibility of food poisoning in such cases of severe oesophagostomiasis. Fortunately, however, this does not seem to be the case. Mason (unpublished work) in making a very careful study (at this institute) of the intestinal bacterial flora of 23 sheep, was not able to isolate any paratyphoid organisms.

(2) *Irregular, darkly staining (with haemalium-eosin) particles.* These are regarded as the early stages of calcification, which is characteristic of the old nodules.

(3) *Parasites.*—The oesophagostomum larvae may or may not be present in the structureless central portions of the nodule. Sometimes they are found towards the periphery of the nodule. Several nodules in one field may be produced by different larvae or they may be produced by one migrating larva. It seems that larvae, having emerged from their cysts, can migrate through the mucous membrane of the intestine, without leaving much evidence of their presence. Sometimes an eosinophile infiltration of a greater or lesser degree may be produced, but this probably disappears very soon after the larvae have again returned to the lumen of the intestine. If, however, the migrating larvae penetrate the muscularis mucosae, then an intense reaction is elicited from the tissues concerned, in the form of an active eosinophilic infiltration. It is possible that such wandering larvae may again pass through the muscularis mucosae and the mucous membrane into the lumen of the gut finally to complete their life history. However, the

probability is that they will wander aimlessly about in the depths of the intestinal walls, and according to Carne and Clunies Ross (1932) and others may even be found in the mesenteric glands and such remote organs as the liver, having as it were lost their sense of direction. Most of them will be destroyed and it seems extremely likely that the macroscopic nodules so characteristic of nodular worm infection, indicate as stated by Monnig (1934), some form of resistance on the part of the host to infestation and represent larvae which are being or have been destroyed, in their wanderings. On post-mortem examination, one sometimes finds very numerous adult worms in the lumen of the bowel when only a few nodules are present. This tends to indicate that in what can be described as the normal development of the parasite, viz. primary migration into the mucous membrane, encystment against the muscularis mucosae and secondary migration back into the lumen of the gut, without penetrating the muscularis mucosae—very little disturbance of a permanent nature is produced in the walls of the intestine. In the abnormal development of the parasite, instead of migrating back through the mucous membrane into the lumen of the gut after it emerges from the cyst, it penetrates the muscularis mucosae and elicits an intense tissue reaction as a result of which the nodules form. That something of this kind may take place is supported to some extent by the observation, which is not infrequently made on post-mortem examination, that numerous nodules are present, when no adult parasites may be found in the lumen of the intestine itself. However, in this connection one should not loose sight of the possibility, indeed even the likelihood, that in some of these cases, the adult worms may have been got rid of by medicinal treatment, as a result of diarrhoea, or may even have died of old age. It is more than likely, that there must be some definite factor (physical, biological, chemical) which induces the parasite to refuse the path of least resistance, from the muscularis mucosae, through the mucous membrane, to complete its development in the lumen of the gut and to prefer to penetrate the barrier, in the form of the muscularis mucosae, only to loose itself in the depth of the intestinal wall.

Towards the periphery of the nodule there is evidence of fibroblastic activity, and in some cases a good deal of fibrous tissue is present. In addition to the nodules themselves, the migrating larvae in this case inflicted considerable mechanical injuries to the intestinal walls, having produced large blood tracts in the mucous membrane the submucous and other tissues right up to the serosa.

(12 *Days after Infection—continued.*)

Lamb No. (2). *Specimen* 6582.—Large intestine. In portions of the intestine pronounced lesions are seen macro- and microscopically. The lesions may involve the entire thickness of the intestinal wall and in places there is actual perforation. In such cases there are multiple, discrete and circumscribed lesions which vary in size up to 2·5 mm. in diameter, on the serosa. (Plate XIII). Centrally there is a well marked pit or depression which is the actual hole where the bowel wall has been perforated. The borders of the lesions are prominent and raised above the surrounding normal tissue surface. The whole lesion has a rather characteristic elliptical shape. In places there is a fibrinous deposit on the serosa (fibrinous peritonitis).

PLATE XIII.—Specimen 6582. 12 days after infection.

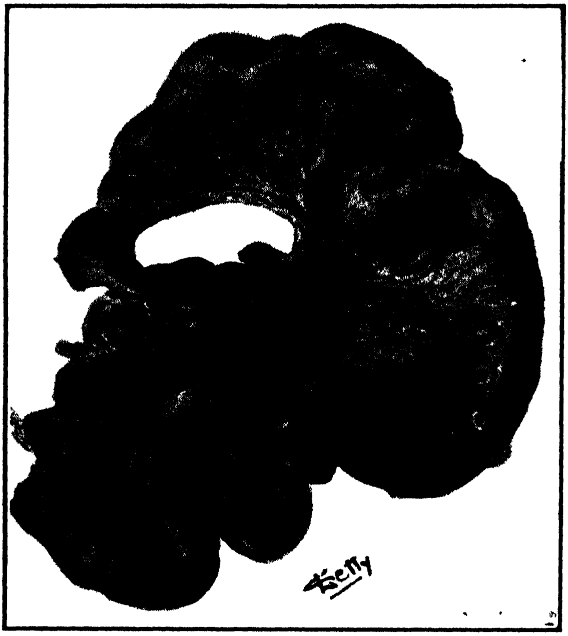


Fig. 1.—Large intestine with perforation due to Oesophagostomum larvae.

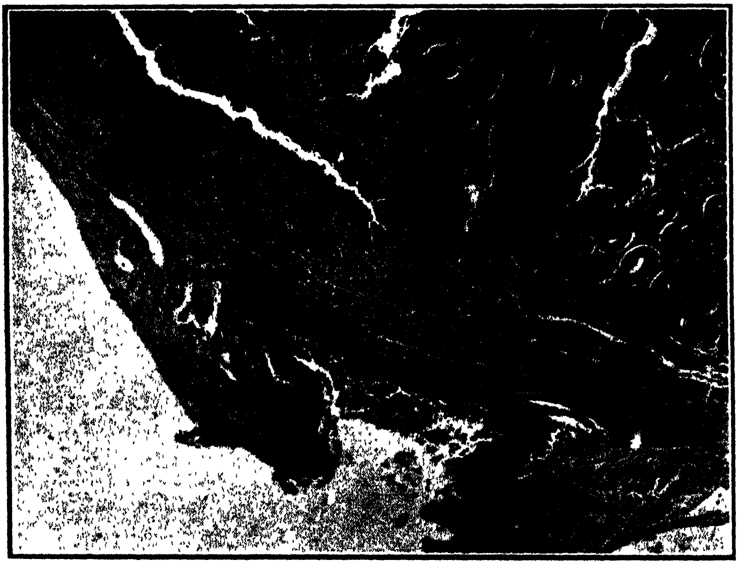


Fig. 2 (38×).—As Fig. 1.

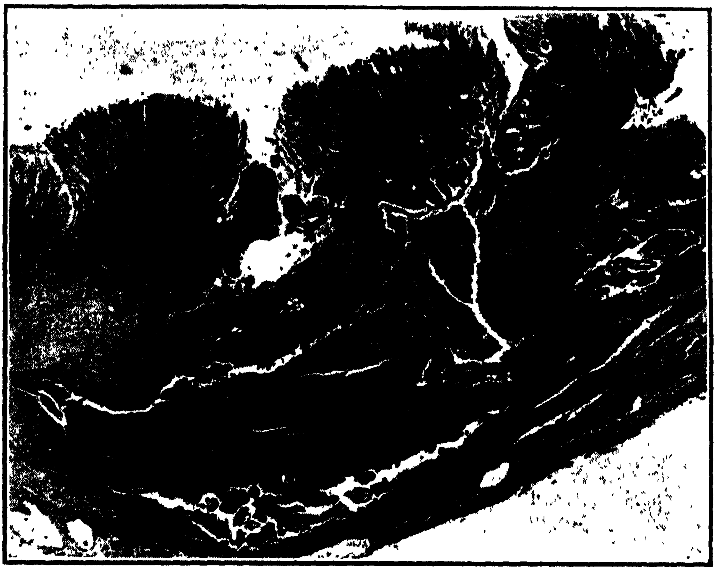


Fig. 3 (20×).—Nodule in wall of intestine, showing wandering larvae and ulceration.

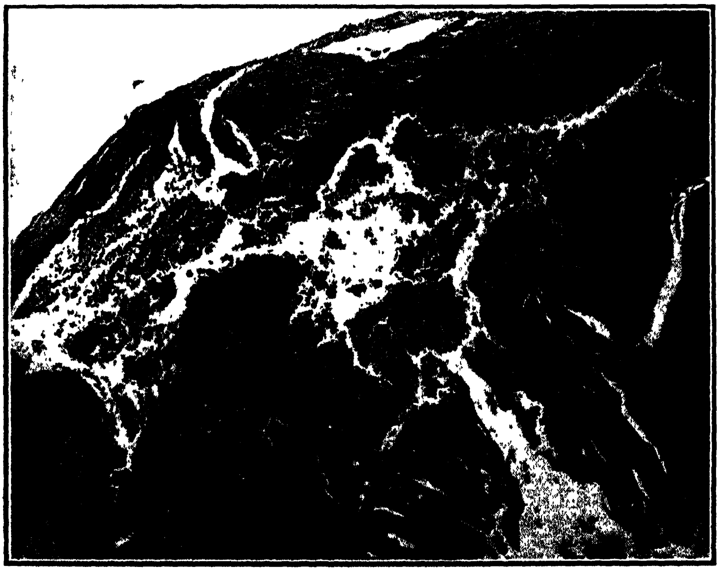


Fig. 4 (38×).—Tracks of wandering larvae and ulceration.

Microscopically the lesions are similar to those described for lamb No. (1), except that in these sections perforation of the intestinal wall is clearly seen (Plate XIII). The tracts of the wandering larvae in the submucosa, represented mainly by masses of eosinophiles, some of which are partially or completely disintegrated, appear in some sections to be quite independent of the tracts of the larvae which have produced the perforation. However, if one carefully follows out a complete series of sections, it is clearly seen that the tracts referred to communicate with one another and in this way it was possible to study the wanderings of the larvae from the mucous membrane through the submucosa and muscular layers, until complete perforation through the serosa has occurred. In such cases one must expect to find larvae in the peritoneal cavity. This observation was actually made by Veglia (1923), confirmation of which will be reported subsequently in this paper. The effect of such perforation would depend largely on the presence of bacteria which could have been introduced from the lumen of the intestine. In this case bacteria were seen to be present in the lesions, where perforation had occurred and it is more than likely that the fibrinous perintonitis already referred to was caused by these organisms. In one case, which will be referred to in greater detail later, when discussing the pathogenesis of the disease, the *bacillus pyocyaneus* was isolated in practically pure culture from the peritoneal cavity. In this case death was due to peritonitis as a result of perforation of the intestinal wall subsequent to infection with oesophagostomum larvae. At the moment a considered opinion as to the extent that peritonitis and other bacterial complications may be important factors in natural cases of oesophagostomiasis cannot be given. However, to judge from the fact that bacteria were repeatedly shown to be present in lesions of the disease during the course of this study, bacterial complications in oesophagostomiasis of sheep would seem to be rather more important, than is suggested by the admittedly very guarded statement of Taylor (1935) that " it seems highly probable that the injuries caused to the bowel wall by parasitic worms in general do not play any important part in bringing about bacterial infection from the lumen of the intestine ".

15 *Days after Infection.*

Lamb No. 64. *Specimen No.* 6583.—Nodules in which parasites may or may not be present, are seen in the small and the large intestine. They are situated in the mucosa, submucosa and in some cases large nodules measuring 1 × ·5 cms. are situated beyond the inner circular muscular layer, completely replace the longitudinal muscular layer and cause a bulging of the serosa. In the mucous membrane there are actually ulcers present and in the caseous mass which completely replaces the glandular epithelium, parasites are seen, probably just on the point of passing into the lumen of the intestine. Therefore, it would seem that as long as the wandering larvae remain alive, they may at any time find their way back into the lumen of the intestine. Obviously it is impossible to devise experiments which would supply reliable evidence as to the proportion of larvae which may successfully migrate back into the lumen of the intestine, after having penetrated the muscularis mucosae. My own opinion is that the majority of them are destroyed when this occurs.

Numerous bacteria can be recognised readily on microscopic examination and in addition to eosinophiles, there are also numerous neutrophiles. In these cases one is therefore really dealing with a worm nodule, which is complicated with abscess formation. In order to get further information concerning the constituents of the nodules, smears were made from nodules present in sheep which passed through the post-mortem room at this institute in the ordinary way for routine post-mortem examination. In this way a number of smears were examined from fourteen different sheep. The smears were stained by Pappenheim's May-Grünwald-Giemsa method. Whilst in some cases there was complete destruction of the cellular elements so that they could not be differentiated in any way, in the case of others, the cells were easily recognised. In nearly all cases eosinophlies were present. In quite a number of cases there were, in addition, numerous neutrophiles, not infrequently bacteria were present. These included cocci and various types of bacilli, e.g. short rods, bipolar organisms, and organisms having terminal spores. This is confirmatory evidence that in quite a number of cases, the nodules are infected with bacteria, but in actual practice this does not occur to the extent which is suggested by the statement of Cameron (1933) that " Bacterial contamination is very common and the simple helminthic nodule becomes converted into a pyogenic abscess ". The usual practical experience is that animals showing symptoms of severe oesophagostomiasis make a remarkable recovery if the adult worms are removed by treatment, which cannot have any significant specific effect on any bacteria which may be present in the nodules themselves.

Towards the periphery of the nodule, granulation tissue is being formed, and in addition to fibroblasts there are some cells which resemble very strongly epithelioid cells. In some nodules a certain amount of fibrous tissue has been formed. In a number of places the blood vessels immediately around nodules are markedly distended. This may be due to a certain amount of mechanical interference on the part of the nodule, with the free venous drainage of the intestinal wall.

17 *Days after Infection.*

Lamb No. 3. *Specimen* 6584.—Small and large intestine—no encysted larvae were found. Tracts of wandering larvae are seen throughout the wall of the intestine. The nodules themselves show the presence of very extensive central necrosis. In this caseated mass cellular elements cannot be recognised, but the presence of calcium particles indicate the early stages of calcification. In a number of nodules numerous bacteria (cocci and bacilli are present. At the periphery of the lesions there is a rather darkly staining zone in which the nuclei of the cells are seen in varying stages of disintegration. The whole lesion is surrounded by granulation tissue, which in places has already formed adult fibrous tissue. In this tissue eosinophiles are present in abundance. In many of these nodules the wandering larvae are seen cut either transversely or longitudinally. They may be present in the central structureless mass, at the periphery of the nodule or sometimes just outside of them. Up to now no definite evidence of dead parasites has been seen, but in this particular animal there are occasional bodies, in which the normal structure is greatly altered, and judging from their outline there is the possibility that they may be portions of the dead bodies of the parasites.

In the ileum, of which complete cross sections were made, well marked changes are seen in the lymph follicles and in the mucous membrane. In the case of the lymph follicles, the lymphocytes are conspicuous by their absence. Indeed it is only here and there that normal lymphoid tissue can be recognised in portions of the follicles. There are not really well defined lesions in the follicles, but the cellular elements seem to have been drained away and the tissue has undergone a kind of rarification as a result of which gaps have literally been left. On the other hand follicles of the control lamb contain dense masses of cellular elements. No definite statement as to the presence or absence of fluid (oedema) in the lymph follicles of the ileum can be made. Unfortunately the usual regional lymphatic glands in well-developed cases of oesophagostomiasis were not available for examination. In the circumstances it is not possible to say if these changes in the follicles of the ileum are definitely due to the effects of the nodular worms, but it is likely that this is the case. The question now arises as to whether the condition is one of lymphoid atrophy or of lymphoid hypoplasia. There is no evidence that the glands are decreased in size and except for the decrease in the number of lymphocytes, there are no recognisable structural changes. It is not known if the glands are producing lymphocytes or other cells at an increased or decreased rate. If there is hyper-activity in the glands, there should be a lymphoid hyperplasia or hypertrophy, but such compensatory processes may be inhibited or controlled by toxins from the parasites. There is a possibility that toxins may interfere with the normal production of lymphocytes and if that is the case, the condition would be one of lymphoid hypoplasia, rather than that of atrophy of the glands, especially in view of the absence of definite evidence that the glands are decreased in size.

In the case of the mucous membrane there is very extensive desquamation of catarrhal cells, which are easily identified in the exudate in the lumen of the intestine in complete transverse sections. This is interpreted as an acute catarrhal enteritis, probably caused by the parasites after they have returned to the lumen of the intestine at the completion of secondary migration. (Plate XIV.)

In one place a wandering larva is seen in a mass of structureless material, which completely replaces the mucous membrane there. (Plate XIV, Fig. 1.) Its head is just free of this material and it appears to be on the point of entering the lumen of the gut. This is further confirmatory evidence that such wandering larvae may find their way back into the lumen of the intestine and that this possibility exists as long as the parasites remain alive.

PLATE XIV.—Specimen 6584. 17 days after infection.

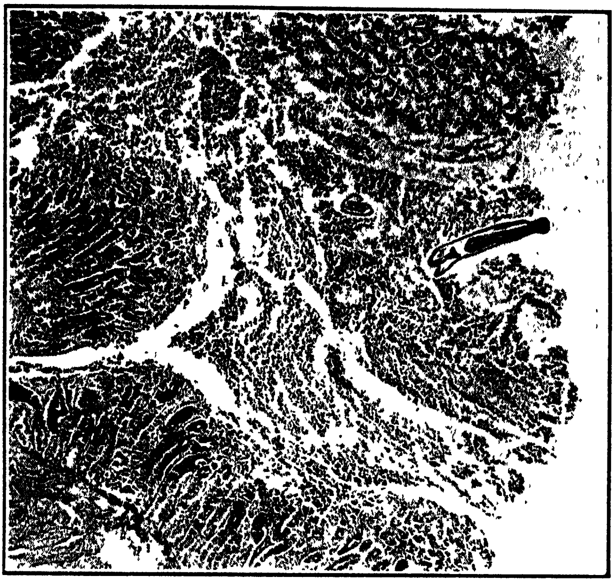


Fig. 1 (35×)—Wandering larva on the point of returning to lumen of intestine.

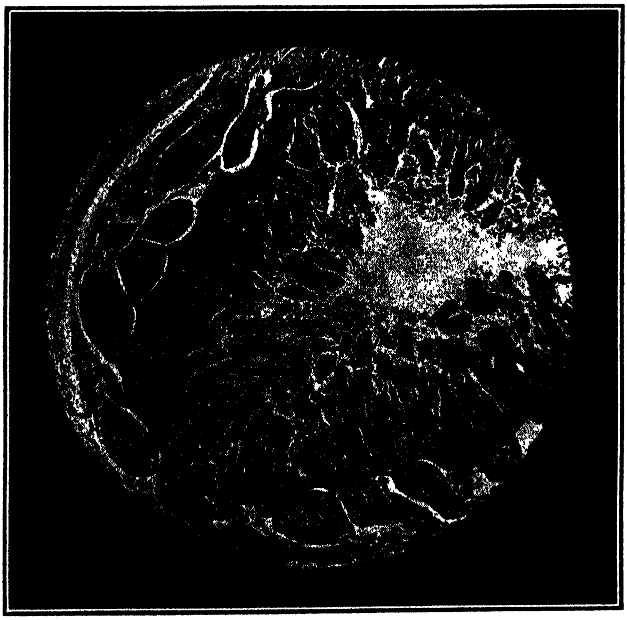


Fig. 2 (25×).—Small intestine, showing catarrhal enteritis.

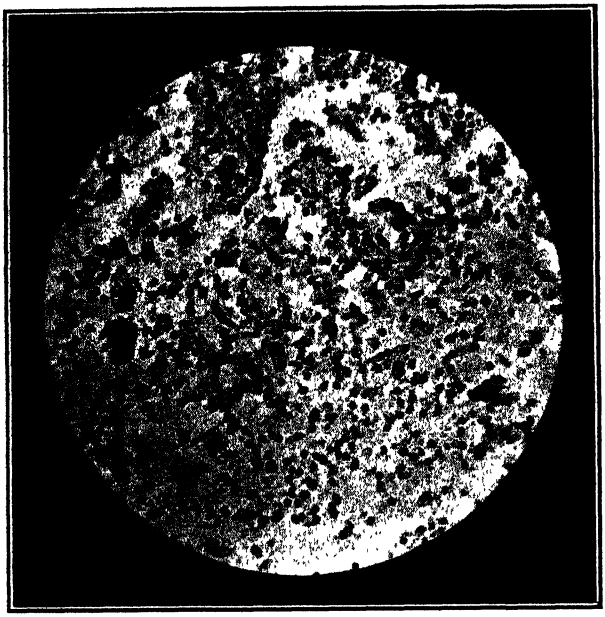


Fig. 3 (220×).—As Fig. 2, showing exudate in lumen of intestine.

Large blood spaces are present, some of which extend from the submucous tissues, through the muscularis mucosae and into the mucous membrane. On careful examination these are seen to be not haemorrhages as they were at first thought to be, but actually distended blood vessels having an endothelial lining. Here again the explanation which suggests itself is a mechanical interference with the free venous drainage of the intestinal wall by worm nodules.

18 *Days after Infection.*

Lamb N. Specimen 6587.—Lesions are present in the small and the large intestine. The nodules are situated mainly in the submucous tissues and their structure is very similar to that of nodules of the previous case (17 days after infection). In some nodules, bacteria and/or parasites are present, whilst in others one or the other or both of these may be absent. The bacteria are chiefly bacillary forms in chains, many with spores which appear as refratile bodies all along the chain. In Plate XV, Fig. 1, a cross section of the ileum is shown, in which there is very marked thickening of the serosa. In this thickened portion are numerous bacteria(Fig. 2). These were probably responsible for peritonitis here. The exudate is now being organised and a good deal of fibrous tissue has already been formed. In the same plate, figures 3 and 4, a nodule is shown in which the parasite appears in longitudinal section. Within its mouth capsule there is a mass of cells which have polymorphic nuclei. The cytoplasm of these cells stains pink, and although no eosinophile granules can be recognised it is believed that they are ingested eosinophiles. It is not clear if the parasite is primarily making use of

PLATE XV.—Specimen 6587. 18 days after infection.

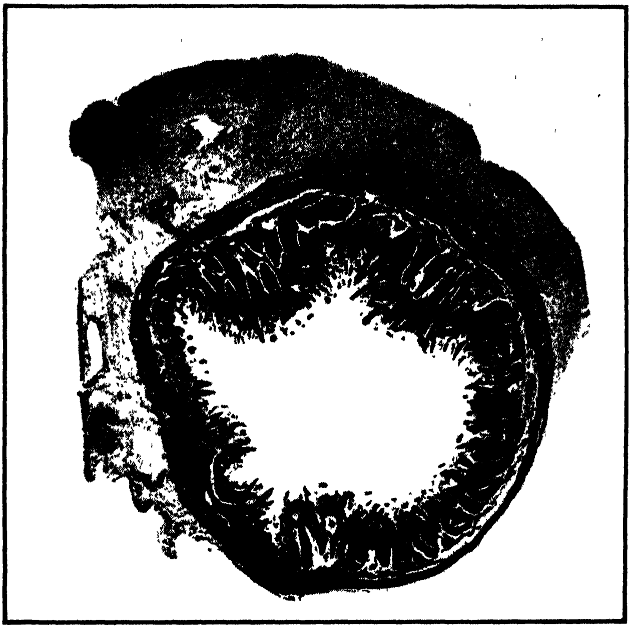


Fig. 1 (8X).—Section Ileum, showing thickened serosa due to peritonitis.

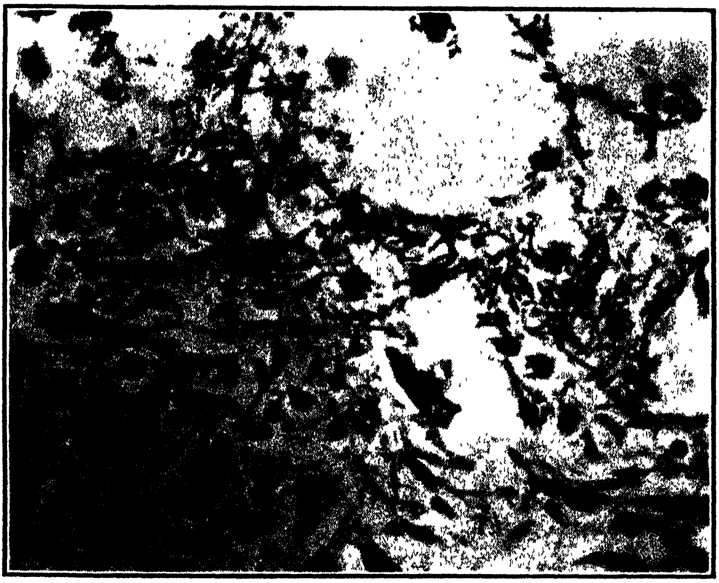
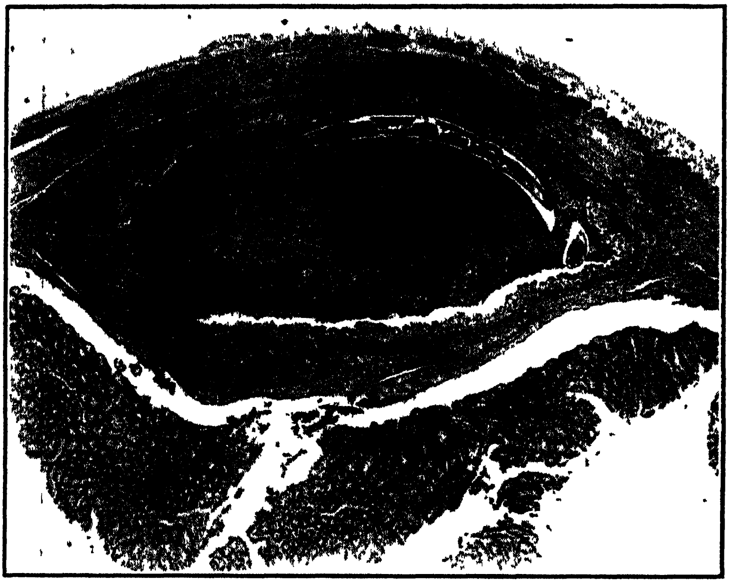


Fig. 2 (750X).—As Fig. 1, showing bacteria at extreme margin of the thickest portion of the thickened serosa.



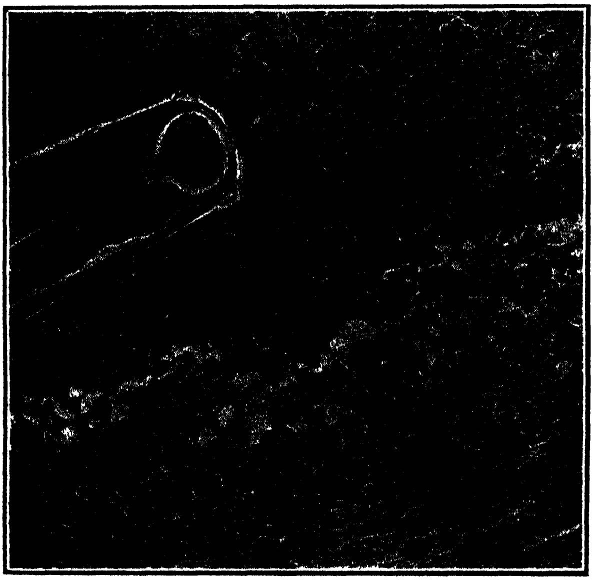


Fig. 3 (25×) and Fig. 4 (220X).—Showing parasite and structure of nodule in the submucosa.

these cells for food purposes, or if it merely devours them in order to protect itself against their attacks. It does not seem likely that the parasite could decrease materially the actual numbers of these cells in this way. To what extent the possibility exists that the ingested eosinophiles have a special biological significance, leading to an immunity of the parasite against their attacks, is at the moment entirely speculative. Should this be the case, the life of these wandering larvae would be considerably prolonged and the damage they inflict would consequently be greater. In several places there are darkly bluish staining (haemalum-eosin) hyaline masses, which lie closely applied to the parasite. An occasional eosinophile is present in the substance of these masses. This is obviously an acid substance which is taking the basic stain. It may possibly be secreted by the parasite itself. Its significance is not understood.

Towards the periphery of the central structureless mass intact eosinophiles and in some nodules probably also neutrophiles can be identified. This is surrounded by a small zone consisting of fairly large cells, having a faintly staining cytoplasm, which sometimes appears to be markedly vacuolated. Some of these cells have more than one nucleus. They are regarded as epithelioid cells and the parasite seems to have made a circular tract all along the epithelioid zone. In the epithelioid zone one sees now for the first time occasional well developed foreign body giant cells. Around this zone there is an accumulation of two kinds of cells mainly. These are eosinophiles and round cells. The round cells are much smaller than the epithelioid cells, amongst which some of them lie. Their nuclei stain rather darkly. These two kinds of cells can almost be said to form another zone here around the epithelioid cells. This zone merges into a zone of granulation tissue, where fibroblasts are readily identified and in places fibrous tissue has already been formed, the whole constituting a capsule. The parasite has now probably become imprisoned within this capsule, and although it may be possible, it seems unlikely, that it would break through this barrier.

Some nodules cause complete destruction of not only the circular but also the longitudinal muscular layers in the wall of the intestine. One can well imagine that in some cases nodules may cause a complete break in the plain muscle of the intestine at a particular place and although as pointed out by Theiler (1921) that this may not be the only cause of intussusception in sheep, it probably is the cause of complete interruption of peristalsis and consequent invagination (reksiekte) in some cases.

There is evidence of hyperaemia and catarrhal enteritis. Not infrequently eosinophiles are seen diffusely distributed throughout the mucous membrane.

21 *and* 22 *Days after Infection.*

Sheep 23 *and Kid* 24. *Specimens* 6589 *and* 6589A.—The lesions (including peritonitis) except that they are rather more advanced. are otherwise very much like those of the previous case. The central caseous mass is relatively large (Plate XVI, Fig. 1) and a fair amount of calcification has occured. An epithelioid zone is present,

PLATE XVI.—Specimen 6589. 21 days after infection.

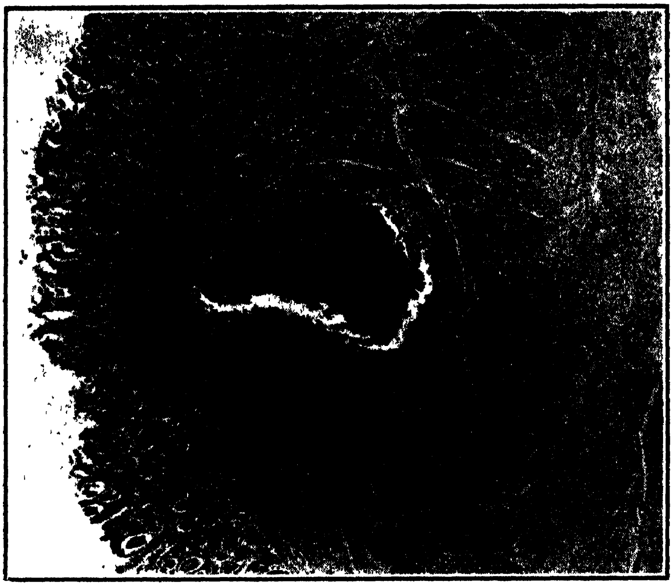


Fig. 1 (25×).—Caseating worm nodule with calcification.

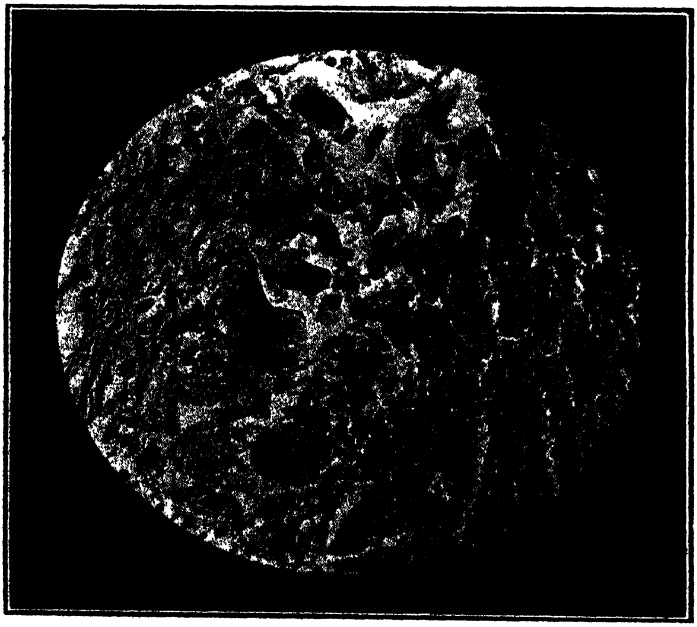


Fig. 2 (220×).—As Fig 1, showing foreign body giant cells.

but this is not very well defined in some nodules and is then to some extent replaced by not infrequent and very typical giant cells. (Plate XVI, Fig. 2.) Many of these have the diffuse distribution of the nuclei, usually associated with foreign body giant cells, but there are some in which the nuclei are present mainly towards the periphery of the cells and resemble then to some extent the Langhans giant cells, usually regarded as typical for tuberculosis. No living parasites could be demonstrated in any of these sections, neither is there any definite evidence of the presence of dead parasites.

28 Days after Infection.

Lamb F. Specimen 6590.—In several places migrating larvae are present in the mucous membrane, but no evidence of encysted larvae was found. The older nodules have more or less the same structure as those previously described. Some of the lesions are very extensive with consequent destruction of a good deal of the wall of the intestine. The nodules may actually project beyond the serosa and extend into the peritoneal cavity (Plate XVII, Figs. 1 and 2), or they may cause a bulging of the mucous membrane into the lumen of the intestine. In such cases there may be an actual ulcer and one can frequently recognise a small macroscopic hole in such nodules in the mucous membrane (actually the nodule may also involve the tissues, deeper than the mucous membrane). In the central structureless mass calcified particles are present. Immediately around the central caseous mass are cells which have a radiating or a palisade arrangement. They are epithelioid cells (Plate XVII, Fig. 3) amongst which one sometimes finds well developed giant cells. The nodules are surrounded by a capsule consisting partly of granulation tissue and partly of well formed fibrous tissue.

PLATE XVII.—Specimen 6590. 28 days after infection.

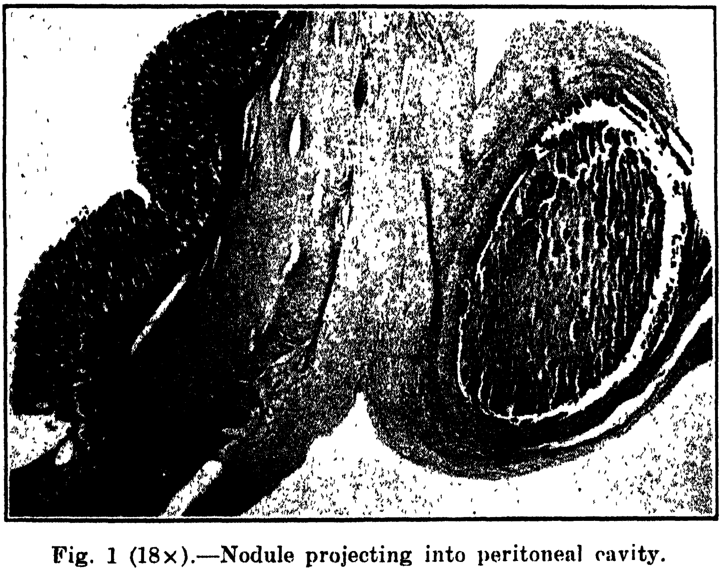


Fig. 1 (18×).—Nodule projecting into peritoneal cavity.

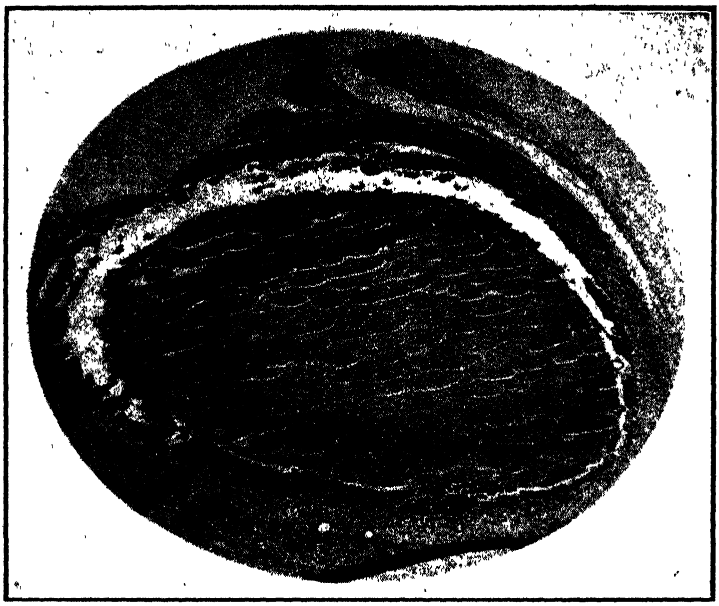


Fig. 2 (30×).—As Fig. 1.

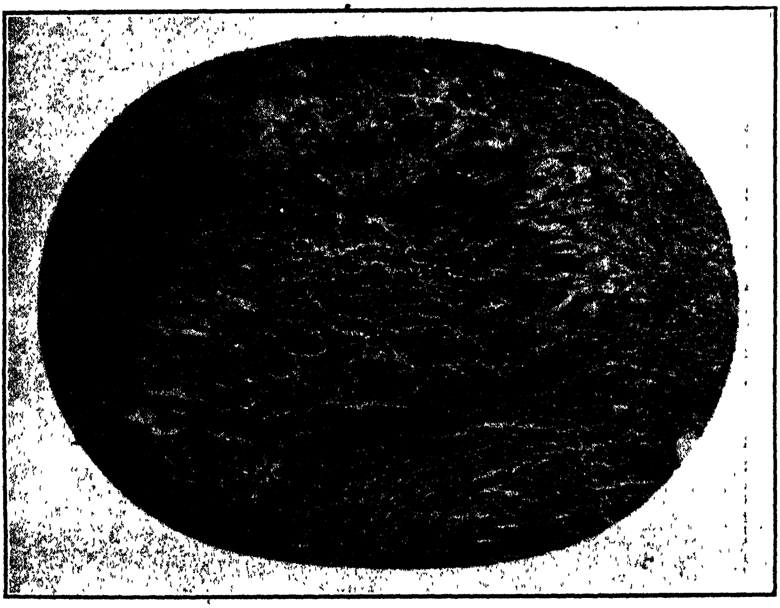


Fig. 3 (120×).—Showing palisade arrangement of epithelioid cells.

30 *Days after Infection.*

Lamb No. 403. *Specimen* 6591.—Lesions are present in the submucous tissues and extend right up to the serosa. Amongst the nuclear debris, polymorphic nuclei can be recognised, but it is not possible to say if they are eosinophile or neutrophile nuclei. Bacilli are present, mostly in the form of chains. There is nothing in the structure of the lesion which suggests definitely its verminous origin; on the other hand it may be an abscess which may be secondary to the verminous infestation.

32 *Days after Infection.*

Lamb 4. *Specimen* 6592.—The usual nodules are present in the walls of the intestine. In some nodules one sees for the first time unmistakable evidence of dead parasites (Plate XVIII, Fig. 1). The disintegrated remains of the parasite lie amongst nuclear debris. Portions of the parasite would seem to be impregnated with calcium. Further calcium particles are found scattered irregularly throughout the nuclear debris. Towards the periphery of the nodule there are not infrequent foreign body giant cells, some of which contain calcified particles. The whole nodule is surrounded by a capsule, which in its inner portions consists of granulation tissue with epithelioid cells and fibroblasts and the peripheral portions consist of fibrous tissue.

PLATE XVIII.—Specimen 6592. 32 days after infection.

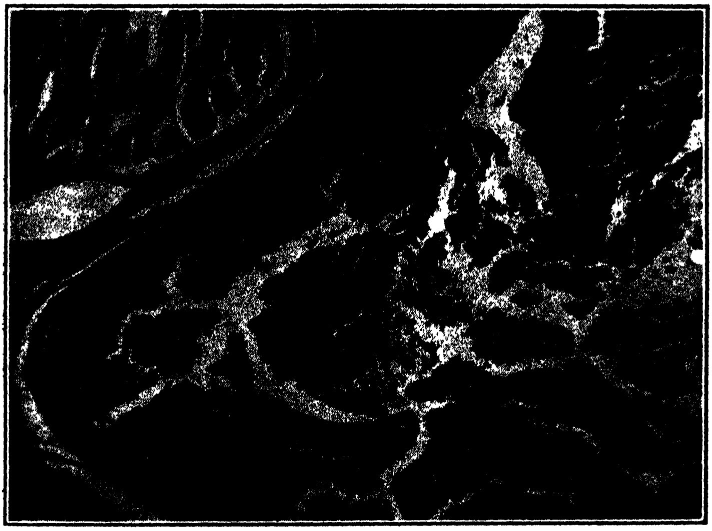


Fig. 1 (65×).—Portions of dead parasite marked by numerals 1.

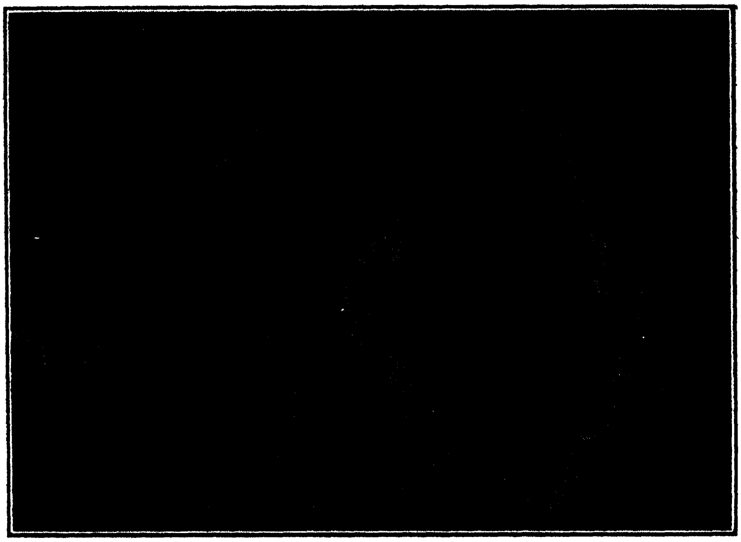


Fig 2 (65×)—Strand of intestinal epithelium at periphery of nodule

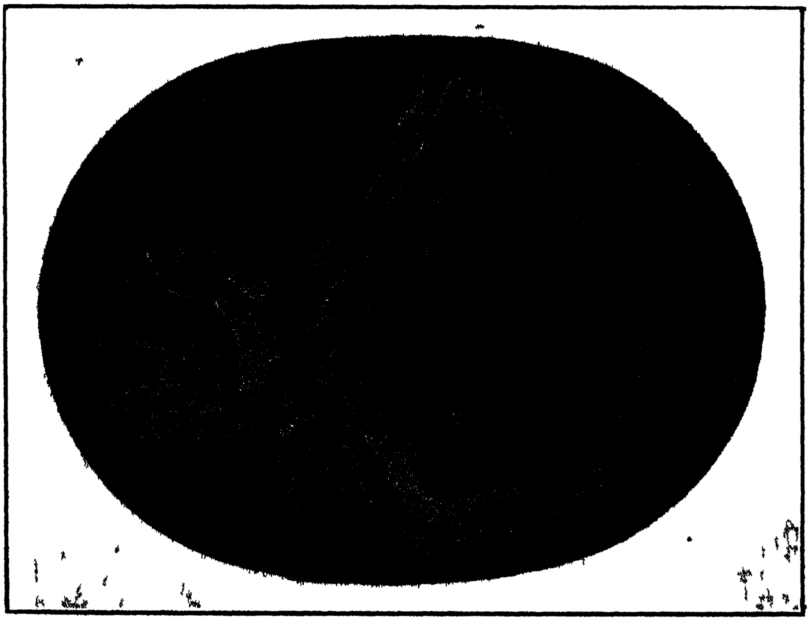


Fig 3 (65×)—Showing structural relationship between epithelial strands around periphery of nodule and mucous membrane

The remains of glandular epithelium from the intestine can be seen towards the periphery of some nodules situated in the submucosa (Plate XVIII, Fig. 2). When the serial sections are carefully examined, it can be seen that these apparently isolated strands of epithelial cells, amongst which goblet cells can be recognised, are structurally connected with the mucous membrane (Plate XVIII, Fig. 3). It seems that such cell groups may easily become detached from their normal structural relationship with the mucous membrane and may then probably give rise to anomalies and even tumours.

40 *Days after Infection.*

Sheep 114. *Specimen* 6593.—Numerous nodules, some of large size, are irregularly distributed throughout the wall of the intestine. The wall of a portion of the intestine is markedly thickened and microscopically this thickening is seen to be largely due to an increase of the plain muscular tissue. Although both the inner circular and the outer longitudinal muscular layers are involved, there would seem to be a greater increase of the inner circular muscular layer. At the same time there would seem to be an increase of fibrous tissue, strands of which are irregularly distributed throughout the plain musculature of the intestinal wall. In the absence of a full post-mortem description of this particular animal, one can only speculate as to the cause of this apparent hypertrophy of the plain muscle. The possibility which suggests itself is, that worm nodules in the walls of the intestine caused a partial obstruction to the free passage of intestinal ingesta and that this stimulated increased peristaltic activity of the plain muscle in the intestinal wall, proximal to the obstruction, with consequent hypertrophy of the parts involved.

45 *Days after Infection.*

Lamb No. 32. *Specimen* 6594.—Numerous and extensive worm nodules are distributed irregularly throughout the walls of the intestine. Some nodules project from the serosa into the peritoneal cavity. Where the nodule is attached to the serous surface of the intestine, its outer portion (capsule) consisting of fibrous tissues completely replaces the outer longitudinal muscle fibres of the intestinal wall, but only involves the inner circular muscular layers very slightly. In most cases the nodules are surrounded by well developed capsules, which in the case of some nodules consist of adult fibrous tissue, whilst in the case of others the capsule is partly fibroblastic. The epithelioid cells, when present, again show a marked tendency to palisade arrangement. Many giant cells and eosinophiles are present. The central portion is mostly structureless, calcification is taking place, but is not very extensive. Parasites can be readily identified in some nodules, but it is not known if they are dead or alive. Bacteria could not be identified in any of these nodules.

56 *Days after Infection.*

Kid No. 44. *Specimen* 6595.—Nodules similar to those of the previous case (45 days after infection) are present. Very many eosinophiles are distributed diffusely throughout the mucous membrane even in portions of the intestine where no nodules are present.

It is not clear if the eosinophile reaction is partly or wholly due to the action of adult worms which may be present in the lumen of the intestine or if it is a reaction which must be associated with the nodules present in the deeper portions of the wall of the intestine, even though such nodules may be situated some distance from parts of the mucous membrane where numerous eosinophiles are also present. No definite evidence of the presence of bacteria was found in the sections examined.

71 *Days after Infection* (2 lambs).

(1) *Lamb* 29. *Specimen* 6596.—Nodules are present in the small and large intestines. At the ileo-caecal valve, lesions are seen in both the ileum and the caecum. Here there is very extensive destruction of the wall of the intestine. In places the entire mucous membrane, the submucosa, the circular and the longitudinal muscular layers are completely replaced by a homogeneous necrotic material, which stains pale pink with haemalum eosin and in which, in addition to very many bacteria, there is present also a certain amount of fibrosis. Where the living and dead tissues meet, there is a zone in which the nuclei of the cells are seen in various stages of necrosis. Beyond this, in the living tissues, there is marked increase in fibrous tissue, in which a fair number of neutrophiles and a greater number of larger round cells are present. Eosinophiles are conspicuous by their absence. The lesion, almost certainly initiated by nodular worm larvae, has now the typical structure of an ulcer, in which not parasites, but bacteria play the principal aetiological rôle.

The structure of worm nodules, uncomplicated by bacteria, in other portions of the large intestine, is well shown in Plate XIX, Figs. 1 and 3. The central structureless portion stains an intense pink colour with haemalum-eosin. There does not seem to be any calcification at all. Immediately around the central portion of the nodule are a number of giant cells. Some of them are typical foreign body giant cells, but most have nuclei arranged around the periphery of the cell, in which respect they resemble to some extent Langhans giant cells. In places there are collections of considerable numbers of eosinophiles. Epithelioid cells and fibroblasts are inconspicuous. The whole is surrounded by a well developed fibrous capsule. Eosinophiles although present in the mucous membrane are not numerous.

(2) *Lamb No.* 13101. *Specimen* 6597.—The nodules themselves are similar in structure to those of lamb 29, except that they are perhaps more extensive (Plate XX, Fig 1 and Plate XIX, Figs. 2 and 3) and that a fair amount of calcification has occured (Plate XX, Fig. 3). In some of the lesions, which as far as can be determined from the examination of serial sections, are not in any way associated with ulcer formation, very numerous bacteria are present. In some of the nodules the remains of dead parasites are seen. The detailed structure can no longer be recognised, but only the bare outlines of the parasite can be seen. In others the parasites are in such a good state of preservation that the probability of their still being alive is very great (Plate XX, Fig. 2).

PLATE XIX.—71 days after infection.

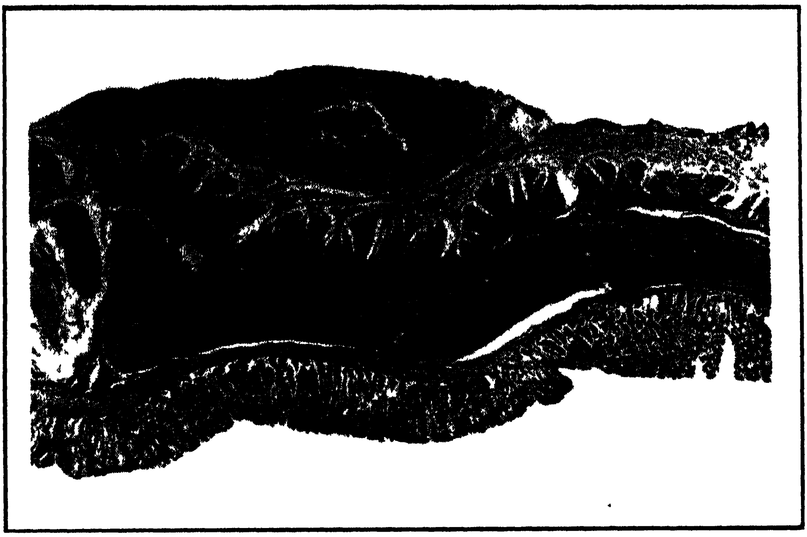


Fig. 1 (18×). –Specimen 6596. Nodule under serosa.

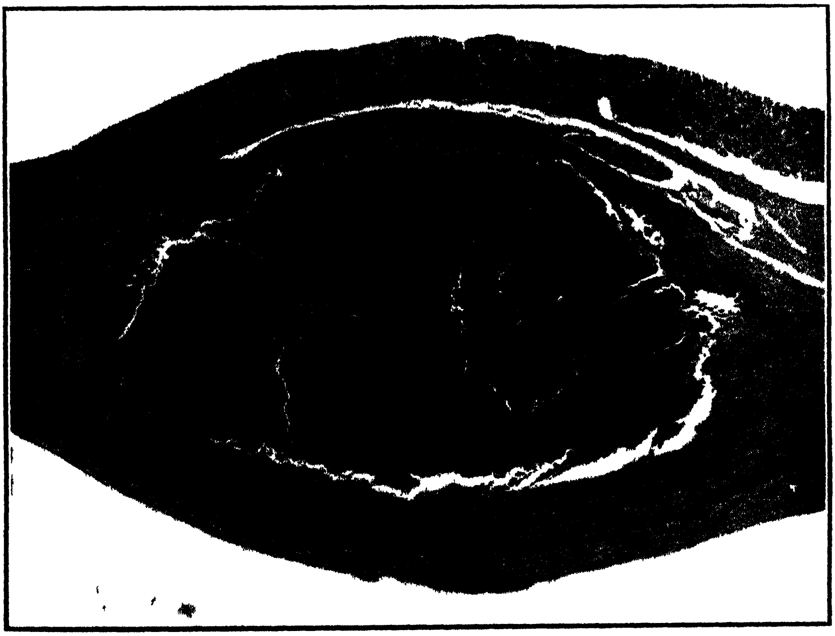


Fig. 2 (18×) —Specimen 6597. Submucous nodule.

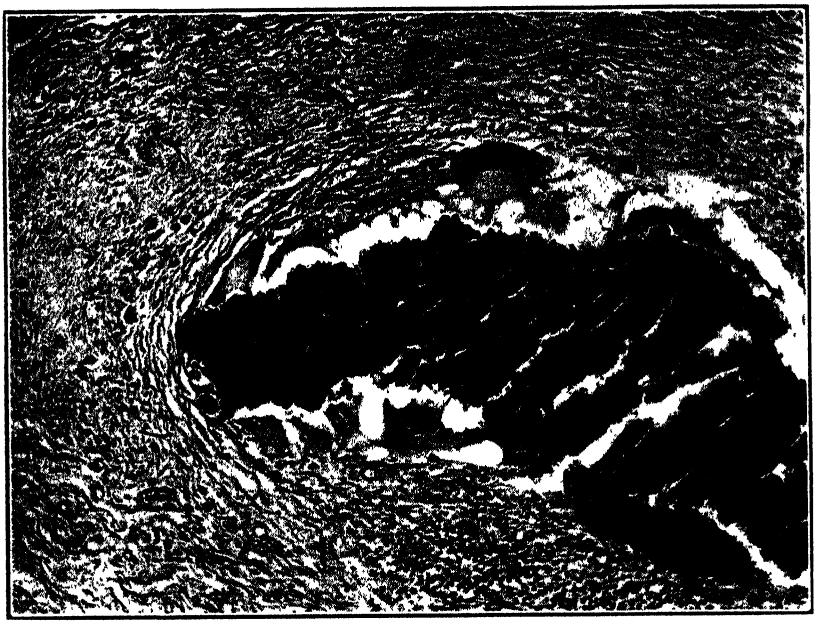


Fig. 3 (120×).—As Fig. 1. Showing giant cells.

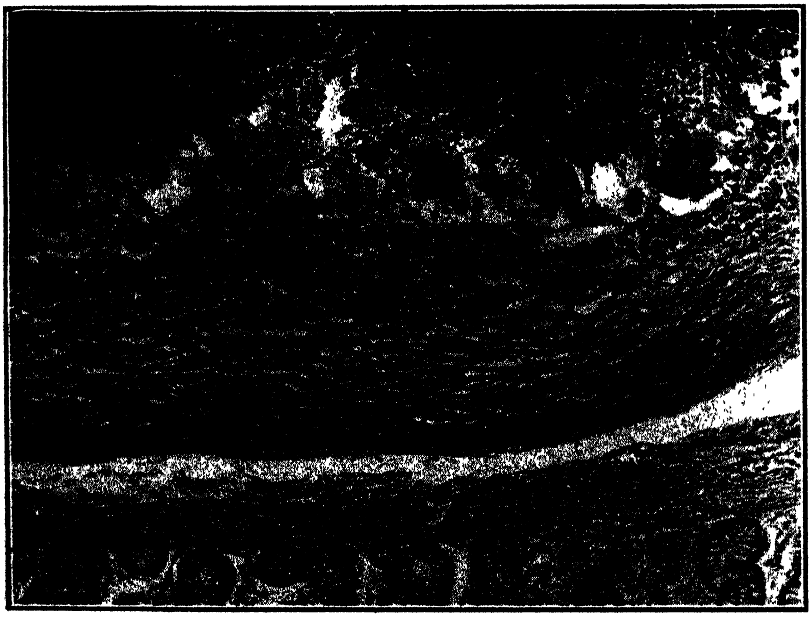


Fig. 4 (120×).—As Fig. 2. Showing giant cells.

PLATE XX.—Specimen 6597. 71 days after infection.

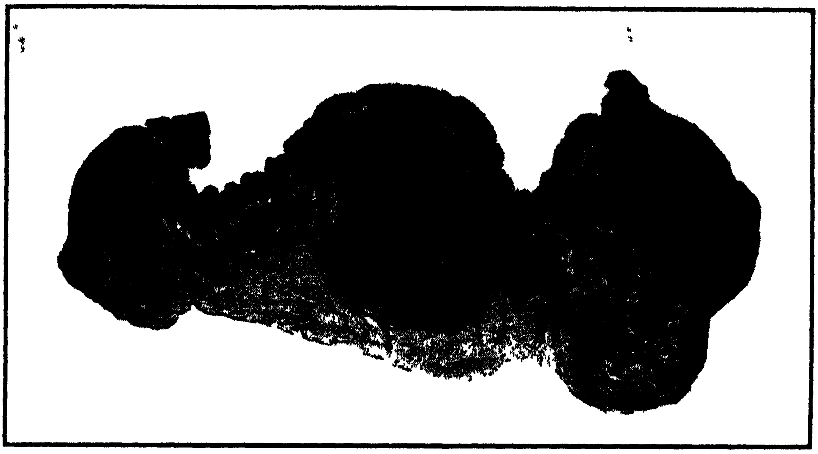


Fig. 1 (6×).—Extensive lesions in the wall of the intestine.

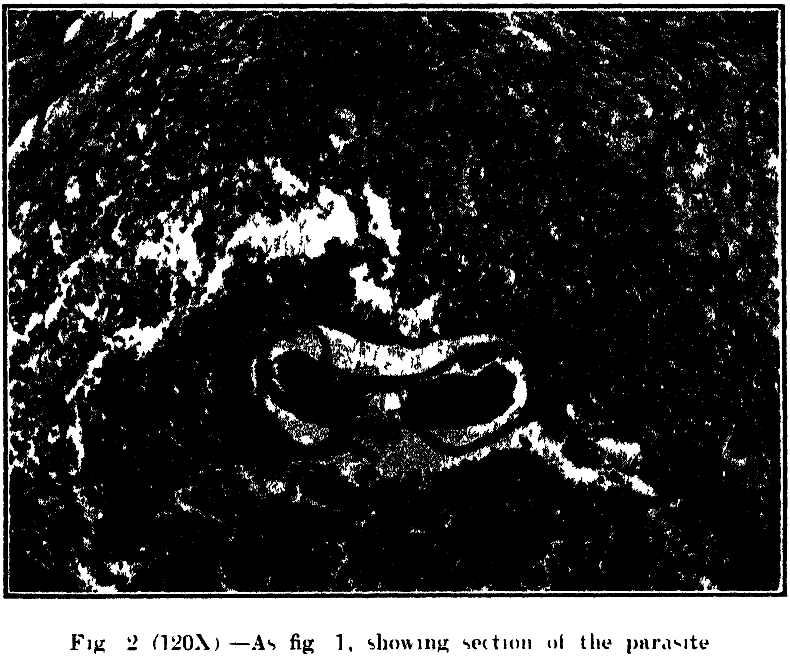


Fig. 2 (120×).—As fig. 1, showing section of the parasite.

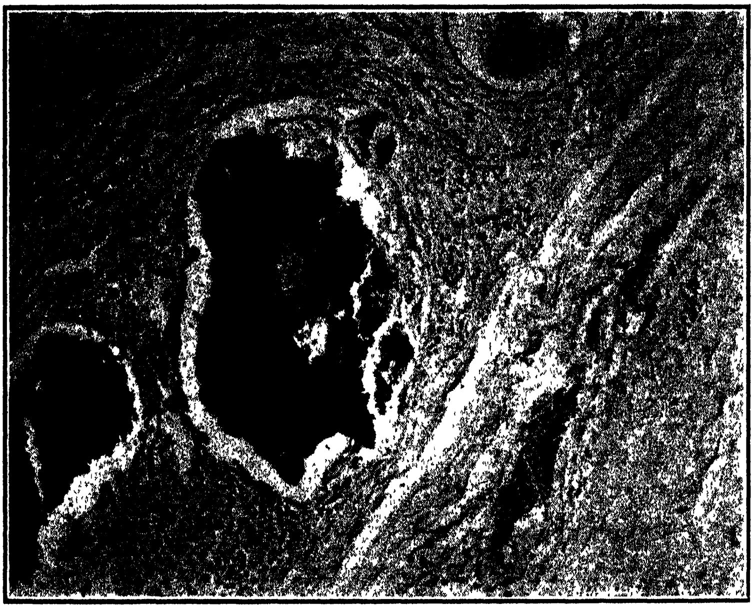


Fig. 3 (120×).—As fig. 1, showing calcification.

106 *Days after Infection.*

Lamb No. 34. *Specimen* 6598.—Well encapsulated nodules are present in the submucous tissues of the small and large intestine. There is no definite evidence of calcification. In some places numerous eosinophiles are diffusely present in the mucous membrane itself. No bacteria can be recognised.

Material from animals known to be infected for exact periods beyond 106 days was not available for examination.

SHORT SUMMARY OF THE PARASITIC LIFE OF THE LARVAL STAGES OF OESOPHAGOSTOMUM COLUMBIANUM IN LAMBS.

(1) 12-24 Hours after Infection.

Twelve hours after infection larvae are demonstrable in the mucous membrane. During this time primary parasitic migration may take place, producing an eosinophilic reaction in the mucosa and sometimes lesions like those of the early stages of a haemorrhagic enteritis.

(2) 24 Hours to 4 Days after Infection.

The larvae encyst against the muscularis mucosae. The muscularis mucosae seems to be merely a convenient mechanical obstruction to the further penetration of the larvae into the wall of the intestine.

The cyst wall consists of a delicate membrane which in a microscopical section has a threadlike appearance, and the nature of which could not be determined with certainty. The larva is coiled within the cyst, in which, in addition to the larva, there are red cells and sometimes structureless, pink staining (with eosin) fluid, which probably consists in part, if not entirely, of haemolysed red cells. Any eosinophile attack directed against the encysted larvae, if at all present, is slight. It does not seem as if the encysted larvae carry bacteria which produce pathological disturbances in the cysts themselves. Two kinds of cysts can be distinguished, viz. (1) those in which there is very little tissue reaction, with only slight fibroblastic activity and (2) those in which greater fibroblastic activity is present, resulting in the formation of a well defined capsule. Larvae in such cysts may be imprisoned there as suggested by Veglia (1923), but no evidence of dead larvae was ever found in the mucous membrane.

(2) 5 DAYS AFTER INFECTION *et seq.*

Secondary parasitic migration takes place. In one case an empty cyst was found three days after infection indicating that in some cases secondary parasitic migration may commence somewhat earlier than was thought to be the case by Veglia (1923). This migration may occur (*a*) normally and (*b*) abnormally.

(*a*) *Normal secondary parasitic migration.*—The fourth stage larva emerges from its cyst. The muscularis mucosae is a mechanical obstruction to its deeper penetration into the submucosa and the parasite is directed through the mucous membrane towards the lumen of the intestine. During its passage through the mucous membrane a secondary eosinophilic reaction (as against the primary eosinophilic reaction, during the primary parasitic migration prior to encystment) takes place, and eosinophiles may be found diffusely distributed throughout the mucous membrane. Although unmistakable evidence that fourth stage larvae in the lumen of the intestine as well as adult worms can produce a diffuse eosinophilia in the mucous membrane, was not found, there is a possibility that this may occur. In the normal migration of the larvae, nodules do not form in the walls of the intestine. In such cases numerous adult worms may eventually be present in the lumen, when only occasional nodules are found in the walls, of the intestine.

(*b*) *Abnormal secondary parasitic migration.*—For reasons which are not understood, the larvae penetrate the muscularis mucosae. Once they have done this, they seem to loose all sense of direction and wander about in the tissues deeper than the muscularis mucosae. During this time an intense eosinophilic reaction is produced and after a time the nodule forms. The very young nodule consists of eosinophiles and the parasite. Within a few days (8 days after infection) some of the eosinophiles disintegrate. This eosinophilic disintegration is the cause of the formation of the central structureless portion of the nodule. However, in the case of some nodules, bacterial activity may contribute to the formation of the central structureless portion of the nodule. Later on calcification may take place. Towards the periphery of the nodule granulation tissue and intact eosinophiles are present. The granulation tissue consists of fibroblasts and epithelioid cells. Later on giant cells are seen and eventually a well developed fibrous capsule is formed.

In wandering about in the walls of the intestine, the larvae may produce very marked destruction of the normal tissues, leading to physiological disturbances associated with a partial stenosis or even interrupted peristalsis (invagination). However, it seems that very marked tissue destruction may take place in the wall of the intestine deeper than the muscularis mucosae, with very little nutritive disturbances, provided no or only very few adult worms are present in the lumen of the intestine. If, on the other hand, numerous primary and secondary migrating larvae produce gross changes in the mucous membrane, bacterial invasion and bacterial complications can readily take place.

During the wanderings of the parasites one of a number of things may happen. (1) It may by good fortune, after repenetrating the muscularis mucosae, find its way back to the lumen of the intestine to complete its development. (2) The tissues may succeed in imprisoning the parasite, which then dies there. It is almost impossible to prove, but, if as is believed, this is the most usual course of events, then the old nodules in the intestine would represent the graves of the parasitic cemetery in the walls of the intestine. (3) The parasite may perforate the intestine and pass into the peritoneal cavity, or may, according to Hall, Carne and Clunies Ross and Mönnig, already quoted and other authors, even pass to remote organs such as the liver, where they eventually die. If the infestation is such that sufficient destruction of the mucous membrane has occured, with consequent bacterial invasion, a fatal peritonitis may take place, with perforation of the intestinal wall. This may explain the hitherto unrecognised cause of some cases of peritonitis of sheep, not infrequently met with in South Africa.

PATHOGENESIS OF OESOPHAGOSTOMIASIS.

In order to understand the pathogenesis of the disease a brief survey of the outstanding clinical features is necessary. There is diarrhoea, accompanied by a very marked emaciation and cachexia. No morphological changes in the red cells, usually associated with anaemia, are present. There are usually no clinical respiratory or circulatory disturbances. The worms actually cause the death of the animal, but exactly how they do this, is not understood. The immediate cause of death is frequently ascribed to toxaemia and exhaustion. At post-mortem one usually finds: adult worms in the large intestine; nodules may be numerous or infrequent; ulcerative or other forms of enteritis may or may not be present; very marked muscular atrophy; marked serous atrophy of fat with emaciation; atrophy of the spleen; degenerative changes of the liver.

In discussing the pathogenesis of the disease the primary, as well as the secondary, factors concerned, will be referred to, viz.: (1) Toxins. (2) Haematology. (3) Complications: (*a*) Those of a specific nature—bacterial infection—enteritis, peritonitis, etc.; (*b*) those of a mechanical nature: (i) destruction of normal tissue with consequent nutritive and other disturbances; (ii) intussusception (reksiekte).

1. TOXINS.

In unpublished work Mönnig and the writer made an attempt to reproduce the symptoms of oesophagostomiasis by repeated injections into susceptible sheep of extracts made from worms collected from sheep which died at Onderstepoort. The results were entirely negative. There is no direct experimental evidence that toxins are mainly or even partly responsible for the symptoms of this disease. However, their presence has been inferred mainly on circumstantial evidence. One meets with fatal cases of the disease in which relatively few nodules are present and in which no evidence of complications such as enteritis is present, in spite of many adult worms in the large intestine. In such cases, the emaciation, general atrophy and advanced degenerative changes of a fatty nature in the liver, seem to be due to toxic principles, derived from the worms themselves. However, there is also the possibility that toxic materials may be derived from the damaged mucous membrane. Whipple quoted by Hewlett (1923) isolated duodenal loops in dogs, with rapidly fatal results in 36-72 hours time. Similar loops from the large intestine were only slightly toxic. If these results in dogs can be applied to sheep, there would seem to be a possibility that toxic substances may actually be formed from the mucous membrane of the large intestine as a result of physiological or other disturbances due to the action of the nodular worms. In severe infestations when the symptoms are most marked, such toxic substances can be formed along the entire length of the large intestine, (as against experimental loops of Whipple) where the worms are present; and even though only a small amount of toxin is formed, its cumulative effect over a period of weeks or months may be quite marked.

2. HAEMATOLOGY.

As far as is known a systematic examination of the blood of sheep suffering from a pure infestation of nodular worms, has not been recorded. Fourie (1931) referred to the difficulties experienced in producing a progressive and fatal disease with amongst others, pure faeces cultures of *Oesophagostomum columbianum*. Nevertheless, with the assistance of Veglia a few such cases were produced and these are the cases which will mainly be referred to in so far as the anaemia is concerned.

The technique employed is exactly that referred to by Fourie (1931). However, in more recent work, it was sometimes found that considerable difficulty was experienced in counting the red cells, as a result of macroscopic agglutination which took place when blood from some sheep infested with oesophagostomiasis and ancylostomiasis, was diluted in Hayem's solution in the ordinary way for counting. Such agglutination was almost completely eliminated, in the few cases which were available for study by heating the Hayem's solution slightly before the blood was added to it. Agglutination will not take place subsequently if the diluted blood is allowed to cool down to room temperature. However, agglutination which has taken place in cold Hayem's solution will not be broken down appreciably by subsequent slight heating. The detailed results from one sheep, the blood of which showed macroscopic agglutination in cold Hayem's solution, are reproduced below.

A.				B.				C.			
Dilution in Cold Hayem's				Dilution in Warm Hayem's.				(B) But Allowed to Cool Down.			
54	60	62	60	59	45	45	45	53	42	47	54
72	60	71	59	41	53	59	38	41	47	54	52
73	68	61	44	47	53	59	36	45	48	52	52
64	69	49	51	52	54	51	49	55	47	46	50
263	257	243	214	199	205	214	168	194	184	199	208
	9·77				7·86				7·85		

A sufficient number of cases of this type of agglutination was unfortunately not available for repeated observation, but to judge from the above case it would seem that although the total counts in (B) and (C) correspond almost exacly, the distribution is much more uniform in the warm Hayem's diluting fluid which was subsequently allowed to cool down before counting (C).*

Haematology of Sheep 14512 *and* 15970 (*Pure Infection Oesophagostomiasis*) and 11899 (*Mixed Infection* Oesophagostomum columb. *and* Haemonchus contortus).

Twelve worm free sheep were infected with faeces cultures of nodular worm larvae. Of these only three (14512, 15970, and 11899) developed a progressive disease from which they died. On post-mortem examination death was found to be due to a pure infestation of *Oesophagostomum columbianum* in sheep 14512 and 15970 and a mixed infestation of *Oesophagostomum columbianum* and *Haemonchus contortus* in sheep 11899. The haematological observations concerning these three sheep are recorded in Table 1, Graph 1 (sheep No. 14512), Table 2, Graph 2 (sheep No. 15970) and Table 3, Graph 3 (sheep No. 11899).

Haematology of Sheep 14512.

The exact age of this animal is not known, but it was under six months. Infection was commenced on the 28th July 1926, at the rate of 200 larvae every second day until a total of 1,600 larvae had been given.

Faeces cultures were positive on the 7th, 9th, 29th, and 30th September 1926. On the 2nd October 1926, the animal was in poor condition and had diarrhoea. On the 11th October 1926, the condition of the animal was very poor and it was weak. The animal died on the 15th November 1926.

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* The statistical analysis of the above figures made by Mr. A. P. Malan (Statistician) confirmed this.

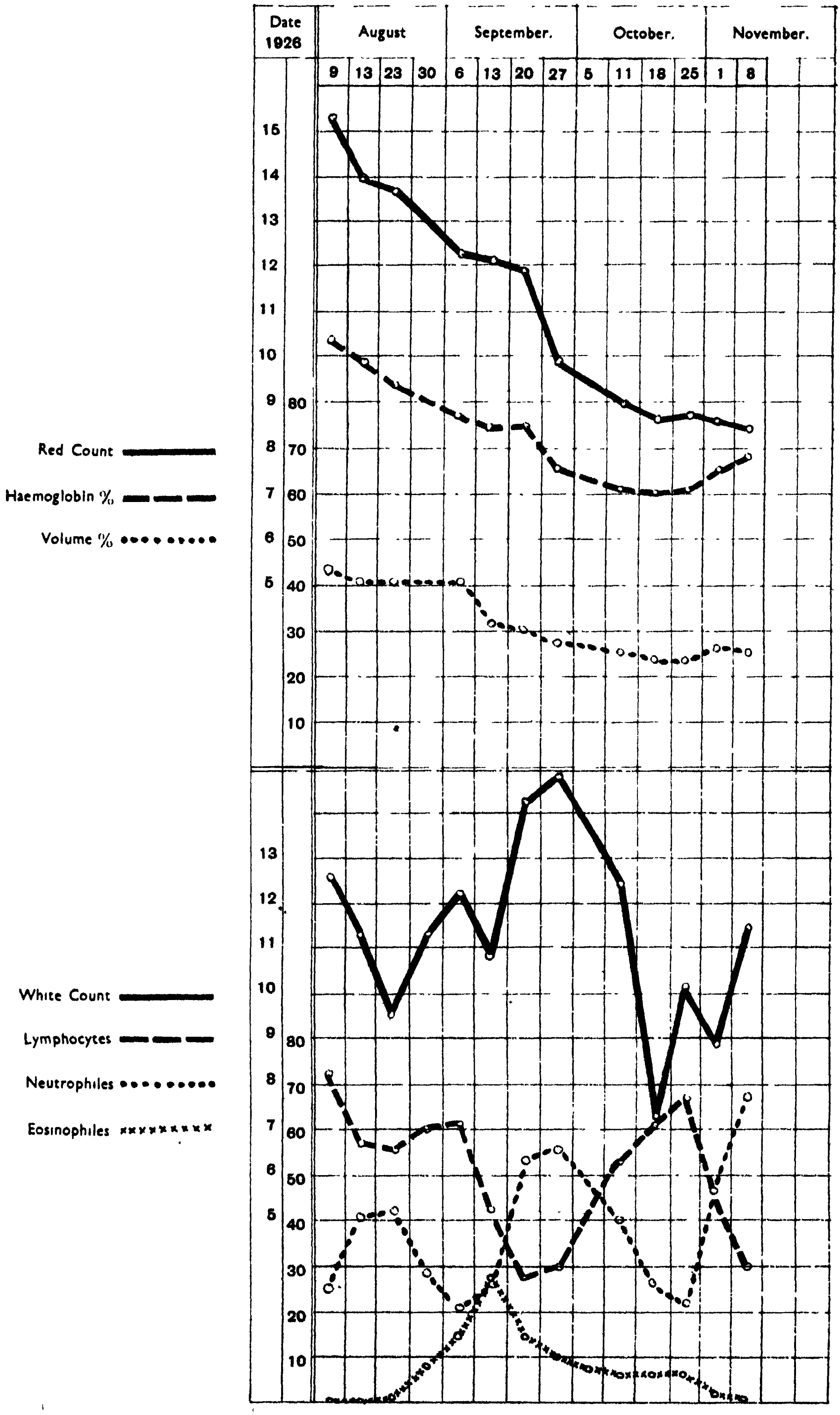
TABLE I.

Sheep No. 14512.

Infection commenced 28.7.26 *at the rate of* 200 *Oesophagostomum larvae every second day until* 1,600 *had been given. Infection completed* 11.8.26

Date.	Source.	R.C.	R.P.	Hglb.	W.C.	L.	M.	N.	E.		Remarks.
9.8.26......	Jugular........	15·3	44	93	12,700	72	2	26	—	—	
13.8.26.....	,, ...	14·0	41	89	11.300	58	1	41	—	—	
23.8.26.....	,, ...	13·7	41	84	9.600	57	—	42	1	—	
30.8.26.....	,, ...	12·3	—	—	11,300	60	2	29	8	1	
6.9.26......	,, ...	12·3	40	78	12,200	61	3	21	14	1	
13.9.26.....	,, ...	12·1	32	75	10,800	43	3	26	27	1	
20.9.26.....	,, ...	11·9	30	75	14,300	28	2	53	14	3	Loosing condition.
27.9.26.....	,, ...	9·9	28	66	14,900	30	—	56	10	4	
5.10.26.....	,, ...	8·5	—	—	14,300	33	3	57	7	—	Condition poor (diarrhoea).
11.10.26....	,, ...	9·0	26	61	12,400	53	—	40	6	1	
18.10.26....	,, ...	8·7	24	60	7,200	61	1	27	6	5	
25.10.26....	,, ...	8·8	24	61	10.100	68	3	22	6	1	
1.11.26.....	,, ...	8·6	27	66	8,900	47	1	47	2	3	
8.11.26.....	,, ...	8·4	26	72	11.500	30	1	68	1	—	

GRAPH 1.—SHEEP 14512.



Changes in the Red Cells.—There is a continuous decrease in the number of red cells almost up to the time of death. It is not clear to what extent this decrease in the number of red cells may be due in part to a normal decrease which may take place in the blood of young animals as they grow older. Fraser (1929/30) refers to variations in the number of red cells of the sheep of different age groups. Unless one can make systematic haematological observations on the blood of a sufficient number of lambs from birth and until they are adult, no reliable conclusions as to the changes which may occur in the number of red cells in sheep as they grow older can be drawn, particularly in view of the variations which are known to occur in the number of red cells of the same sheep as pointed out by Wirth (1931) and Fourie (1931).

The animal still had more than eight million cells per c.c. of blood when it died. This can be a more or less normal count for some sheep. No morphological changes usually associated with an anaëmia were present in the red cells. Obviously, therefore, there is no primary haemopoietic or other disturbance in which the number of red cells can be regarded as deficient (clinical anaemia) in spite of the oligocythaemia which is admittedly present. Any changes in the decrease of the haemoglobin and the percentage volume of red cells, seem to be entirely dependent on the decrease in the number of red cells already referred to.

The oligocythaemia may be due to a diminshed efficiency in the normal function of the haemopoetic organs, which may also be involved in the general atrophy characteristic of the disease. If this is the case the toxins may interfere with the normal regenerative changes which are constantly taking place in the haemopoetic system, with the result that the replacement of worn out cells does not occur normally and a gradual decrease in the number of red cells is then inevitable. There is no direct experimental evidence in support of this view. Furthermore, there is no appreciable morphological differences between the cells of this animal in the earlier stages of infection (9.8.1926) and at the time of death. If there is any atrophy of the bone marrow, it is conceivable that the bones themselves would be subject to similar changes in this disease and although a special examination of the bones was not included in this study one would expect that any defect of the bones such as brittleness would be revealed by a predisposition to fractures. This is not a clinical feature of the disease. Nevertheless, it is possible that atrophy of the haemopoietic system, resulting in oligocythaemia, but not to the extent of clinical anaemia, may similarly affect the bones themselves to a moderate degree only, so that in actual practice a predisposition to fractures is not present.

Changes in the Leucocytes.—The figures presented in Table 1 and Graph 1 loose a good deal in value on account of the fact that preinfection counts were not made and in view of the likelihood that a number of larvae may already have commenced and some even completed their secondary parasitic migration, when the first counts were made 12 days after infection was commenced.

There would appear to be a definite leucocytosis on the 20th and the 27th of September (more or less two months after infection). This is caused mainly by an increase in the number of neutrophiles.

During the next two weeks there would almost seem to be a collapse in the total number of leucocytes, affecting mainly the neutrophiles, an observation which tends to support the view in favour of a possible atrophy of the blood forming tissues previously postulated, particularly as this occured at a time when a similar kind of collapse in the number of red cells took place. During the later stage of the disease the neutrophile counts are definitely high, but the total number of leucocytes do not reach the same high levels as on the 20th and 27th of September. Not knowing the preinfection normal counts, it is useless to calculate the absolute numbers of neutrophiles and lymphocytes etc. This is unfortunate, as there may merely have been a relative neutrophilia, which possibly could have been accounted for by a lymphocytic leucopaenia as a result of a possible greater atrophy of the lymphoid tissues. Probably the neutrophilia indicates that bacterial complications are taking place and if there is a certain amount of atrophy of the blood-forming tissues, the neutrophile counts will not reach the same high levels as would be the case with a normally reacting bone marrow.

Eosinophiles.—There is a very steep ascent in the eosinophile curve which reaches its peak (27 per cent.) six weeks after infection was commenced, and then subsides gradually until the death of the animal. The peak of the eosinophile counts occured at a time when the total leucocytic counts were relatively low. If these total counts can be regarded as more or less normal, the possibility which suggests itself is that previous to, or, during the time that the eosinophile counts were increasing, there was no significant reaction in the myeloid tissues and that the eosinophiles were produced elsewhere possibly in the lymphoid tissues of the intestine. This is not incompatible with the view previqusly expressed that an eosinophilia may indicate that the active tissue verminosis has been completed, when these cells are temporarily released into the circulation. The subsidence of the eosinophiles may be due to either (1) a diminished or (2) an increased effect of the parasitic factor.

(1) *The Diminished Effect of the Parasitic Factor.*—The last dose of infective larvae was administered just about a month before the peak of the eosinophile counts occured. During this time the larvae should all have completed their normal secondary parasitic migration, so that from this source there should be no stimulus for the production of eosinophiles. Also it is possible that during this time there may be a decrease in the effects produced by abnormal secondary parasitic migration, but no definite statement to this effect can be made as it seems that until the parasite dies or finds its way back into the lumen of the gut, the abnormal parasitic migration may continue indefinitely. It is not known when the parasites will die and the factors such as food, age, general hygiene of the sheep etc., which may influence the life of the parasite, during parasitic migration are not understood.

(2) *The Increased Effect of the Parasitic Factor.*—This may cause atrophy of the tissues which produce the eosinophiles. During the two weeks subsequent to the time that the peak in the eosinophile counts was reached, there is a very definite increase in the total

leucocytes, accounted for exclusively by an increase in the number of neutrophiles. This speaks against a myeloid atrophy during this time. Similarly the increase in the differential lymphocytic counts which occured during the latter part of October may be regarded as evidence against atrophy of the lymphoid tissues. But here again the differential counts may be a very unreliable index of the true state of affairs as actually there may have been a neutrophile leucopaenia during this time, accounting for the relative increase in the number of lymphocytes.

The haematological changes can be summarised as: Oligocythaemia but no clinical anaemia; eosinophilia which subsides gradually during the later stages of the disease; neutrophilia probably indicating that bacterial complications are taking place, but there is a possibility that, especially during the two weeks before death, the neutrophilia may be merely relative and possibly due to a lymphocytic leucopaenia.

Haematology of Sheep 15970.

This animal was a control in a wireworm experiment but later (8th February 1927) it was infected with nodular worm larvae at the rate of 5,000 per day until 35,000 had been given. The animal died 13 days after infection and because of the acute course of the disease the haematological observations do not reveal any significant changes, except for a leucocytosis six days after infection and about a week before the death of the animal.

This case has been included here mainly on account of its special pathological interest. It is a two-tooth ewe in fair condition. There are many erosions and even ulcers especially in the mucous membrane of the small intestine. In many places there is perforation of the intestine, particularly the ileum. The perforations as seen from the serosa are discrete and more or less elliptical defects, with raised borders, presenting a pathological picture almost identical to the case previously described (Plate XIII). Where perforation has occurred the omentum is adherent to the intestine. There is a fibrinous peritonitis from which the *Pseudomonas pyocyanea* was isolated in practically pure culture. The caecum shows an acute haemorrhagic enteritis. There are numerous nodules in the small intestine, but only a few in the large intestine. The liver shows well marked fatty degeneration. Unfortunately there is no record of the number of parasites present in the lumen of the intestine, and an examination was not made for the fourth stage larvae in the peritoneal cavity. Here then, is a case in which a young sheep was subjected to a gross infection with nodular worm larvae and in which in numerous cases, there was abnormal secondary parasitic migration, leading to perforation of the wall of the intestine. As a result of the extensive destruction of the mucous membrane and other parts of the intestine by primary and secondary migrating larvae, bacterial infection took place and later caused the peritonitis which was the immediate cause of death.

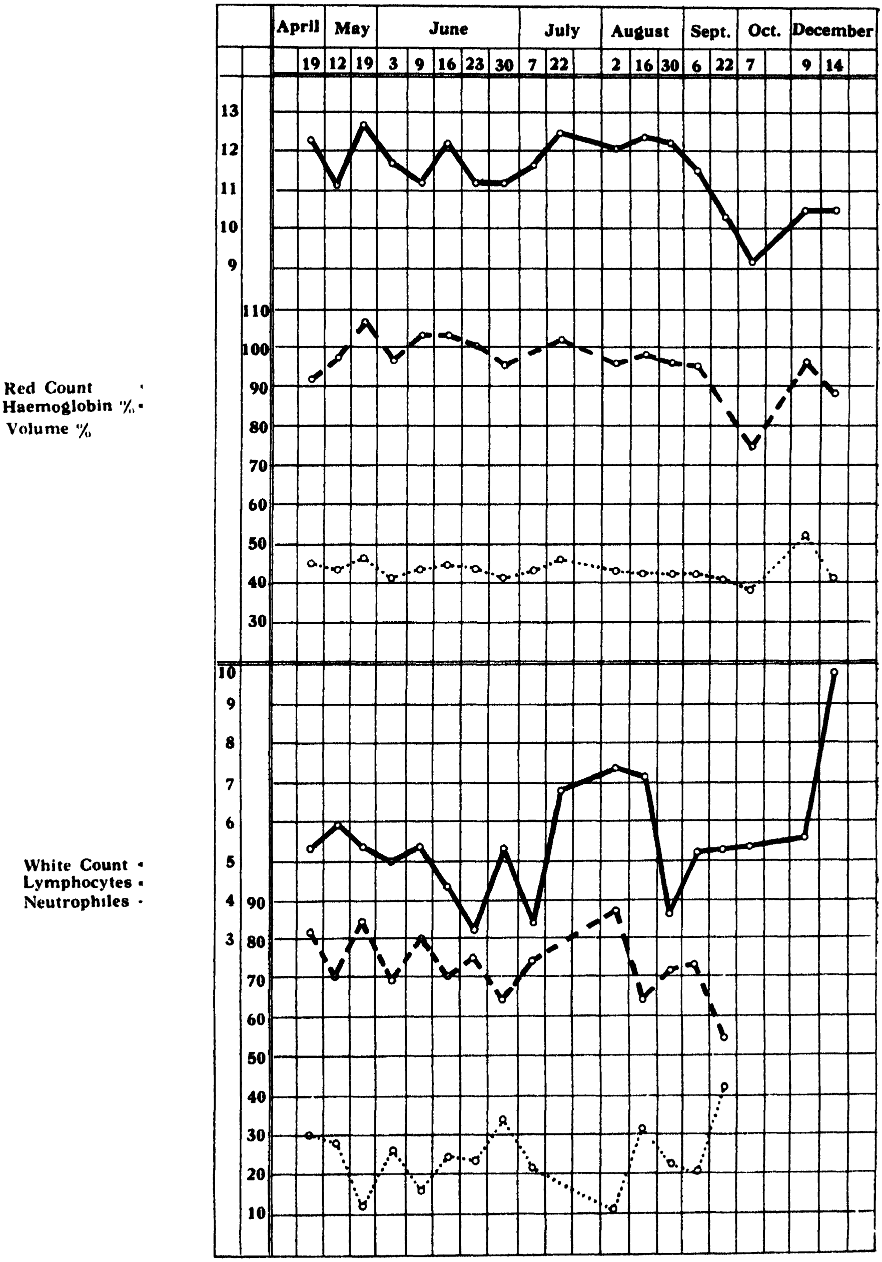
TABLE II.

Sheep No. 15970.

Infection with Oesophagostomum columbianum *larvae was commenced* 8.12.27 *at the rate of* 5,000 *larvae per day until* 35.000 *larvae had been given.*

Date.	Source.	R.C.	R.P.	Hglb.	W.C.	L.	M.	N.	E.	B.	Remarks.
19.4.27.....	Jugular........	12·3	46	91	5,300	81	4	13	2	0	
12.5.27.....	,, ...	11·2	44	98	5,900	70	1	28	0	1	
19.5.27.....	,, ...	12·8	47	107	5,300	85	1	12	2	0	
3.6.27......	,, ...	11·7	41	96	5,000	69	0	27	3	1	
9.6.27......	,, ...	11·2	44	102	5,300	80	0	16	4	0	
16.6.27.....	,, ...	12·2	44	102	4,400	70	1	24	4	1	
23.6.27.....	,, ...	11·2	44	100	3,200	75	0	23	1	1	
30.6.27.....	,, ...	11·2	42	95	5,300	64	2	33	0	1	
7.7.27......	,, ...	11·7	43	—	3,400	74	0	21	1	4	
22.7.27.....	,, ...	12·5	47	101	6,800	Smear	unsatisfactory.				
2.8.27......	,, ...	12·0	43	96	7,400	86	1	11	2	0	200 cells counted.
16.8.27.....	,, ...	12·4	42	98	7,100	64	3	31	1	1	
30·8·27.....	,, ...	12·2	42	96	3,600	71	5	22	1	1	
6.9.27......	,, ...	11·6	42	95	5,200	73	3	20	3	1	
22.9.27.....	,, ...	10·2	47	—	5,300	54	4	42	0	0	
7.10.27.....	,, ...	9·2	38	75	5,400	—	—	—	—	—	
9.12.27.....	,, ...	10·5	42	97	5,600	—	—	—	—	—	
41.12.27....	,, ...	10·5	41	88	9,700	—	—	—	—	—	

Graph II.—Sheep 15970.



Haematology of Sheep 11899.

This case has been included here in order to show the type of blood picture which can be expected with a mixed infection of nodular and wireworms. Infection with nodular worm larvae was commenced on the 16th November 1925, at the rate of 200 or 5,000 larvae every second or third day, until a total of 51,400 larvae had been given. In the course of time, the animal became also grossly infected with wireworms accidentally.

The animal died on the 11th February 1926 and showed on post-mortem examination: Very marked anaemia (hydraemia, paleness of all mucous membranes); cachexia, with serous atrophy of fat; general atrophy of organs and the musculature; degenerative changes of the myocardium, liver and kidney; worm nodules, severe infestation with nodular and wireworms.

On referring to Table 3 and Plate XXI, it will be seen, that the outstanding morphological changes in the red cells are those of poikilocytosis and anisocytosis, and that cells showing punctate basophilia were definitely not present to the extent they occur in most cases of haemonchosis, according to Fourie (1931). Fourie further believes that punctate basophilia in haemonchosis indicates regenerative changes and is a sign of active haemopoiesis. The fact that these cells are present to a slight degree only in this case of mixed infection, may possibly indicate that the nodular worms have caused atrophy of the haemopoietic tissues, as a result of which active regenerative changes which are usually caused by a well developed wireworm infestation, with its resultant anaemia, are now largely dominated by degenerative changes, the atrophied bone marrow being no longer able to respond efficiently to the stimulus for active regeneration.

Haematology of Sheep 18000, 17995, *and* 18344.

In view of the possibility that eosinophiles in a sheep infected with nodular worms may be produced outside the myeloid tissues, total and differential white cell counts were made from blood collected from the jugular, aorta, caudal vena cava, mesenteric veins and, in some cases also, from the portal vein, from two sheep (17995 and 18344) infected with nodular worms, as well as from a control uninfected sheep (18000).

The sheep were infected on 26th September 1927 and blood was collected under anaesthesia ten days later.

In the control sheep eosinophiles were found to the extent of 1 per cent. in the jugular vein, but none were found in the other vessels, when 200 cells were counted.

In sheep 18344 the eosinophile counts were: jugular, 1 per cent; mesenteric, 6 per cent; and aorta, 3 per cent. In sheep 17995 the smears were such that differential counts could not be made, except in the case of the mesenteric vein, where no eosinophiles were found. Although the percentage of eosinophiles is fairly high especially in the mesenteric vein of sheep 18344, no reliable conclusions can be drawn from these results. Such an experiment can supply reliable results only, if total and differential counts can be made at varying periods after infection.

TABLE III.

Sheep No. 11899.

Infection with Oesophagostomum columbianum *larvae commenced* 16.11.25 *at the rate of* 200 *or* 5,000 *larvae every second or third day until* 51,400 *larvae had been given. Infection completed* 23.12.25. *Animal died* 11.2.26.

Date.	Source.	R.C.	R.P.	Hglb.	Viscosity.	W.C.	L.	M.	N.	E.	B.	Remarks.
3.11.25	Jugular	12·4	39	—	—	5,500	62	5	31	2	—	
5.11.25	,,	12·6	34	83	—	4,100	61	1	37	1	—	
9.11.25	,,	12·5	34	80	—	4,800	60	2	37	1	—	
11.11.25	,,	9·8	30	76	—	4,500	53	4	43	—	—	
30.11.25	,,	11·0	35	84	—	6,000	50	3	45	2	—	
25.11.25	,,	11·5	33	86	—	3,700	44	1	55	—	—	
2.12.25	,,	11·1	35	87	—	2,700	58	3	34	3	2	
10.12.25	,,	12·3	35	89	—	3,400	62	1	35	2	—	
17.12.25	,,	10·6	34	84	—	6,100	39	3	54	3	1	
23.12.25	,,	11·7	34	83	—	5,700	32	2	66	—	—	Cells normal.
29.12.25	,,	9·2	28	73	3·1 29	3,600	45	—	50	5	—	Cells normal.
7.1.26	,,	7·3	20	51	2·4 30	3,900	69	3	28	—	—	Anisocytosis.
13.1.26	,,	5·2	16	39	2·1 30	4,200	44	7	43	6	—	cells normal.
15.1.26	,,	5·1	15	38	2·0 29	3,800	42	3	53	1	1	
18.1.26	,,	4·0	12	32	2·0 25	4,100	—	—	—	—	—	Poikilocytosis—Red cells crenated.
20.1.26	,,	4·4	13	32	2·2 26	4,400	32	2	63	1	2	Poikilocytosis punctate basophilia not infrequent.
23.1.26	,,	4·5	13	32	2·1 27	4,600	51	1	46	1	1	Poikilocytosis marked, punctate basophilia infrequent.
27.1.26	,,	3·6	12	28	1·9 28	4,900	49	2	48	1	—	Poikilocytosis marked, occasional punctate basophilia.
29.1.26	,,	4·0	9	25	1·7 28	5,900	48	0	50	2	—	Poikilocytosis, marked anisocytosis (micro- and macro-cytes).
3.2.26	,,	3·9	9	24	1·4 30	3,800	56	1	41	1	1	Poikilocytosis and anisocytosis less marked. No punctate basophilia seen.
5.2.26	,,	3·2	6	21	1·3 31	4,000	40	2	58	—	—	Poikilocytosis very marked. Punctate basophilia not infrequent.
9.2.26	,,	3·3	7	21	1·3	5,300	53	1	46	—	—	Poikilocytosis very marked. Punctate basophilia very rare.

GRAPH III.—SHEEP 11899.

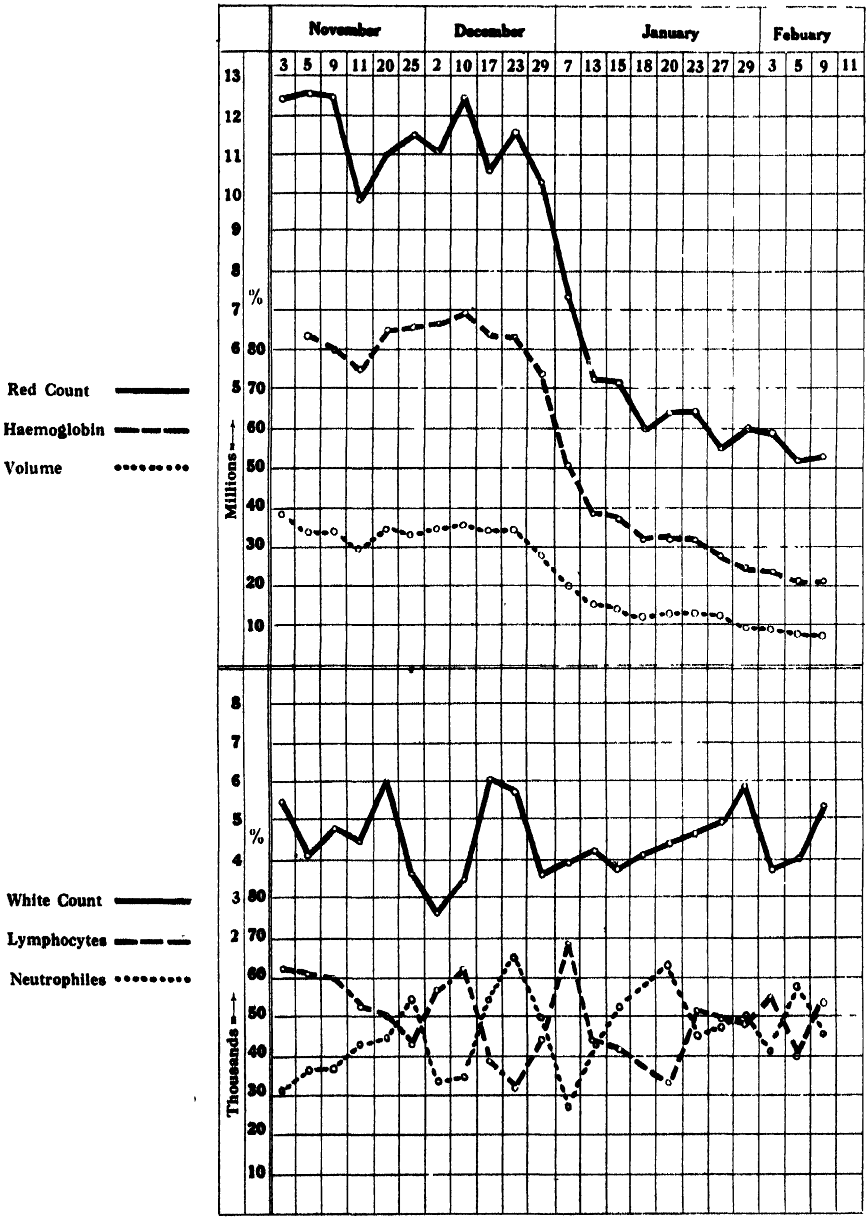


PLATE XXI — Sheep 11899 Mixed infection Haemonchus and Oesophagostomiasis

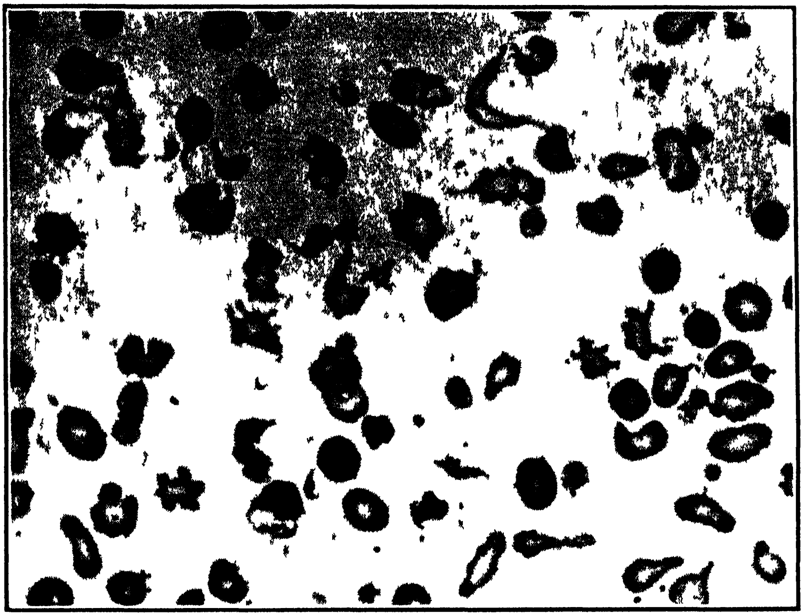


Fig 1 (1250×) —27 1 26 Poikilocytosis

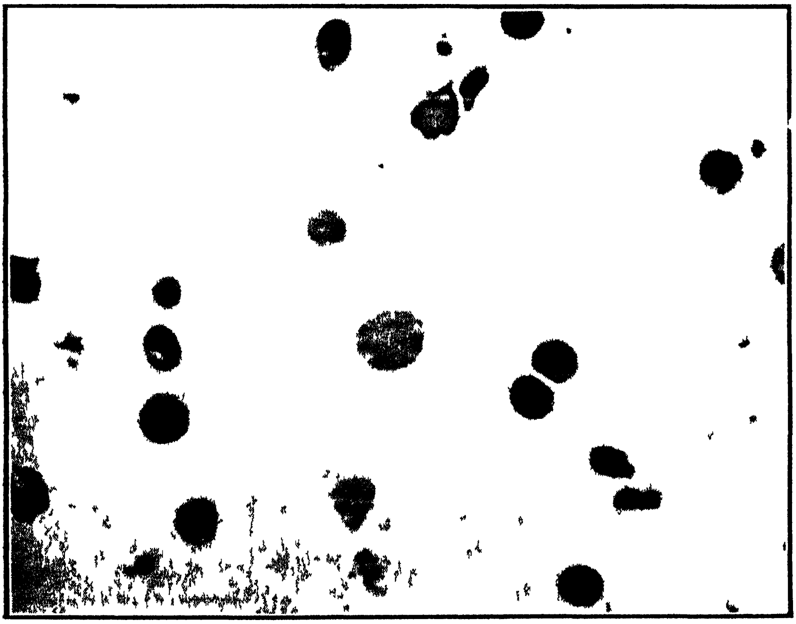


Fig 2 (1250×) —29 1 26 Anisocytosis

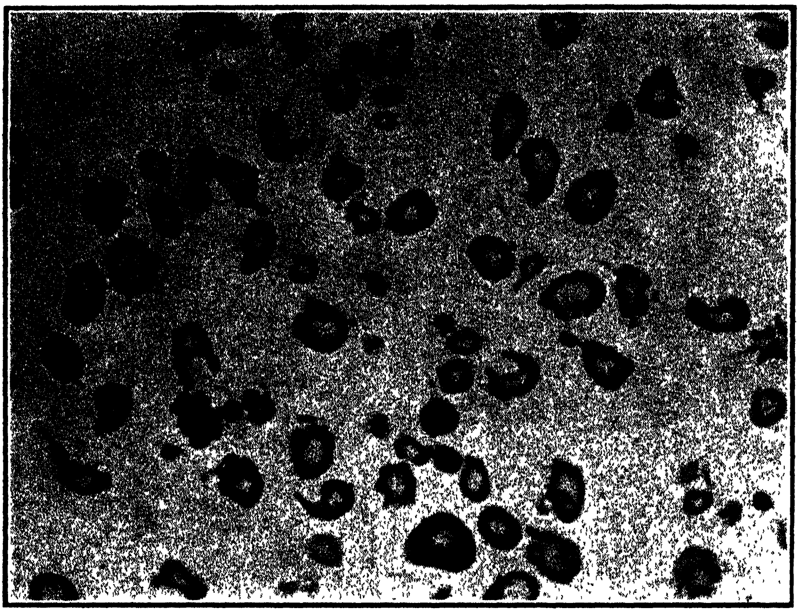


Fig. 3 (1250×).—5.2.26. Poikilocytosis and anisocytosis.

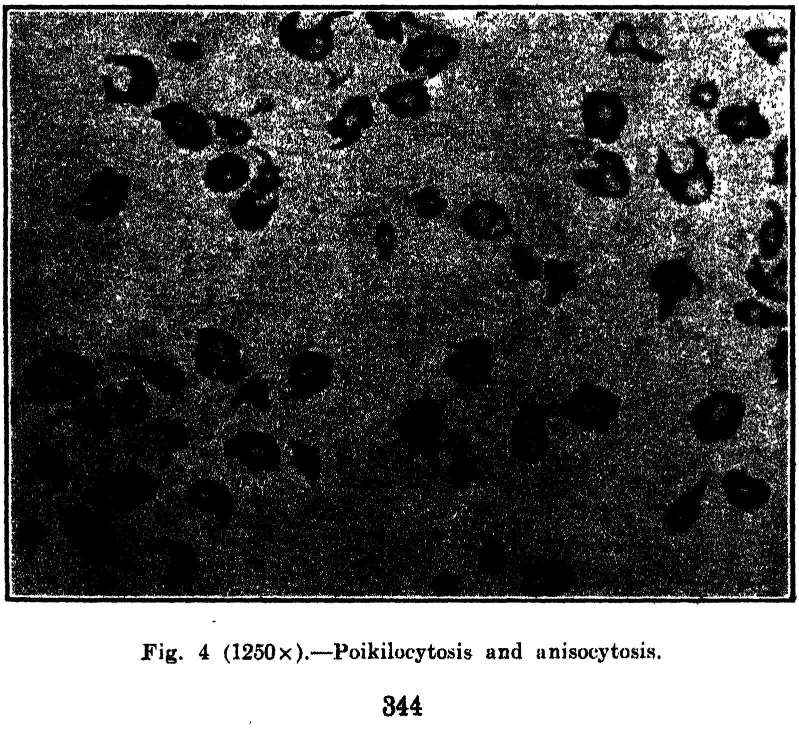


Fig. 4 (1250×).—Poikilocytosis and anisocytosis.

(3) COMPLICATIONS.

(a) *Bacterial Infection.*

As already stated there is no evidence that worms carry pathogenic bacteria with them during primary parasitic migration. The primary migrating larvae may, however, produce such gross anatomical changes in the mucous membrane, especially in severe infestations, that bacterial invasion of the mucous membrane can take place from the lumen of the intestine and produce various forms of enteritis (enteritis superficialis). When the animals are exposed to continuous infection on the pasture, primary and secondary parasitic migration will probably be present in many animals at the same time. In such cases bacterial invasion of the submucosa and other tissues of the intestinal wall deeper than the muscularis mucosae can also occur and produce deep inflammatory processes in the intestinal wall (enteritis profunda) and peritonitis in cases where the serosa becomes perforated. If enteritis is present the condition may be a contributory factor in the production of the diarrhoea characteristic of the disease. However, diarrhoea is a symptom in cases which, on post-mortem examination, were found to be free from bacterial complications. Therefore in such cases it seems that the parasites in the large intestine produce the irritation leading to increased motility of the intestine with consequent diarrhoea. Exactly how they do this (toxins, methods of parasitic feeding, etc.) is not known.

It is very difficult to assess the importance or otherwise of the rôle played by bacteria in oesophagostomiasis. There is no doubt whatsoever that the parasites can and do produce the death of the animal, in cases where bacterial complications are not present, but to judge from the number of cases in which bacteria were shown to be present in the deeper portions of the intestinal wall, during the course of this study, there must be quite a number of cases in which bacteria are a considerable contributory factor in the production of symptoms and of mortality.

(b) *Complications of a Mechanical Nature.*

(i) Hypertrophy of the plain muscle in the intestinal wall was described. It is likely that this may have been due to a partial stenosis, as a result of the presence of worm nodules and it is conceivable that in some cases a complete stenosis may be produced. One would think that the actual destruction of the tissues of the intestinal wall may lead to nutritive disturbances in those cases where very numerous nodules are present. This would not seem to be the case. One repeatedly finds, at post-mortem, very numerous nodules in animals which are in excellent condition. In such cases any bacterial invasion which may have occured has presumably been overcome and any adult worms which may have been present have been got rid of.

(ii) *Intussusception* (*Reksiekte*).—This is a condition which is sometimes met with in South African sheep. Whilst there is no evidence that this condition is always caused by oesophagostomum nodules, there is, in some cases of oesophagostomiasis, a very definite anatomical basis for the development of the condition. In these cases the larvae cause complete destruction of the circular and longitudinal muscle fibres at a particular place during abnormal secondary parasitic migration. As a result of this, peristalsis may become completely interrupted here and invagination may take place.

SUMMARY OF THE PATHOGENESIS OF OESOPHAGOSTOMIASIS.

(1) Although toxins for the experimental reproduction of the disease have not actually been obtained from the nodular worms, there is very strong circumstantial evidence that during the course of the disease poisonous substances are formed and that these can produce the symptoms, lesions and death in the absence of bacterial and other complications.

(2) Although insufficient cases were available for haematological study, there seems to be a possibility that the toxic action of the parasites may also produce a certain amount of atrophy of the haemopoietic tissues leading to oligocythaemia, but a deficiency of the red cells to the extent of a clinical anaemia was not observed.

In some cases there is an eosinophilia. Whether this is due to their increased production in the myeloid tissues and their subsequent mobilization, or, whether they are produced locally in the walls of the intestine and are released temporarily into the circulation at the conclusion of the active tissue verminosis, cannot be stated with certainity.

(3) In some cases bacterial complications producing various forms of superficial and/or deep enteritis, as well as peritonitis, are contributory factors in the causation of symptoms and mortality in the disease.

(4) In some of the lesions there is a definite anatomical basis for the development of partial stenosis and intussusception. Apart from such accidents, the nodules themselves, even though they may be responsible for very extensive tissue destruction, do not seem to produce nutritive or other disturbances, in the absence of parasites in the lumen of the intestine and in the absence of bacterial complications.

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Section VIII.

Photosensitisation.

QUIN, J. I. ... ... Studies on the photosensitisation of animals in South Africa, IX. The bile flow of the Merino sheep under various conditions 351

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Studies on the Photosensitisation of Animals in South Africa.

IX. The Bile Flow of the Merino Sheep under Various Conditions.

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INTRODUCTION.

In a series of articles (1933, 1934, 1935) which appeared under the above general title, various experiments were described in which the main object was the elucidation of the "geeldipkop" problem in South Africa. From the results obtained one was forced to the conclusion that in the symptom complex of this disease two distinct factors had to be considered. Firstly there was the severe generalised jaundice and secondly the acute photosensitisation of unpigmented animals leading to necrosis and subsequent deformity of the facial skin. In considering the problem from different aspects, it was at one time thought possible that the one symptom could be the direct cause of the other. Thus it was thought that some normal biliary constituents, e.g. the bile pigments themselves when present in excessive amounts in the general circulation, could under certain circumstances give rise to photosensitisation. Alternatively is was thought possible that photosensitising principles present in the plant Tribulus or elaborated in the animal body after ingestion of the plant, might be the cause not only of a primary photosensitisation but also of a secondary jaundice through some derangement of the normal liver function. However, numerous experiments conducted in this light have failed to prove—

(1) that any photosensitising principle may in itself be the cause of jaundice, and

(2) that normal bile or any of its constituents when present in excessive amounts in the circulation could give rise to symptoms of photosensitisation.

Moreover as far as could be ascertained from the current literature, no conditions are recorded in which these two symptoms have any bearing on each other.

With regard to the photosensitising factor in geeldikkop, Rimington and Quin (1934) were able to show that the plant porphyrin, phylloerythrin was directly responsible for the symptoms of light sensitivity. It is formed in the digestive canal of many animals feeding on chlorophyll containing materials, and after its absorption into the portal circulation is normally excreted by the liver in the bile and thus passed out with the faeces. Should the excretory function of the liver be deranged, so that increased amounts of bile and thus also of phylloerythrin are present in the circulation, symptoms of photosensitisation may appear at any moment, provided the animals have unpigmented skins and are exposed to sunlight (see article 6 of this series). Furthermore, injections of phylloerythrin into healthy sheep provoke acute photosensitisation without any signs of jaundice. thus acting in the same way as other photosensitisors such as haematoporphyrin, certain fluorescent dyes, and hypericin (Quin 1933, 1934). One was thus forced to conclude that the primary disturbance in geeldikkop caused by Tribulus or by various other plants responsible for this peculiar symptoms complex, consisted in some derangement in the normal bile flow from the liver whereas the photosensitisation was of a secondary and purely accidental nature.

The main problem therefore resolved itself into attempts at elucidating the icterogenesis caused by Tribulus and other materials showing a similar effect. It must however be admitted that so far all attempts have failed in demonstrating an icterogenic principle in these plant materials except in the case of certain species of Lippia and to which reference will be made later on. In Tribulus for example this factor must be of an extremely labile character seeing that the plant may be harmless at one moment and decidedly dangerous soon afterwards.

Furthermore the liver in geeldikkop cases shows very little morphological change microscopically except for the bile staining. Hence the disturbance appears to be largely functional in that it is not regularly accompanied by any of the wellknown structural alterations

In view of these difficulties in explaining the action of Tribulus on the liver it was decided to make a closer study of the normal bile flow in Merino sheep and to ascertain how this was influenced by different factors. As can be gathered from the large volume of literature published, studies on the bile excretory function of the liver have commanded the attention of many workers in different parts of the world. Not only can bile be regarded as one of the most peculiar excretions of the body but through a variety of causes (some known and others unknown) liver derangement may lead to definite symptoms of which jaundice is perhaps the most striking.

In a comprehensive article by Rich (1930) it is pointed out that the development or non-development of jaundice depends on the balance struck between the amount of bilirubin delivered to the liver and the capacity of the liver to excrete it. Thus with a normally functioning liver, excessive amounts of bile pigments are readily eliminated whereas under certain circumstances great difficulty may be experienced in the exretion even of small amounts of bile. The problems of liver derangement are further complicated by the fact that no strict relationship seems to exist between the type and extent of liver damage and the degree of jaundice developed. Thus it is peculiar that in many cases of severe fatty changes of the liver or in extensive cirrhosis the icterus may be only very slight or completely absent. On the other hand as pointed out previously, the liver in geeldikkop may show practically no morphological change and yet the icterus may be most intense.

In studying the bile excretion, regular examination of the blood serum is generally regarded as of the greatest importance since Hymans van den Bergh has demonstrated that not only can the bile pigments be determined quantitatively but also the type of jaundice can be ascertained by the diazo reaction. Beyers (1923), in his monograph on urobilinuria and icterus in herbivorous animals, gives a detailed account of these conditions in the various species. It is pointed out that whereas a physiological bilirubinaemia is present in the horse, no bile pigments are normally found in the blood serum of adult bovines, sheep, goats, pigs and dogs. The serum of the horse, which is normally of a yellow colour, contains 1-2 units of bilirubin, whereas in the other species the serum is usually water clear or only slightly tinged.

This indicates that the equine liver treats bilirubin as a substance with a definite threshold value, whereas in most of the other species it appears to be excreted as fast as it arrives in the liver, i.e. treated as a non-threshold body.

EXPERIMENTAL PROCEDURE.

1. *The Blood Serum in Healthy Sheep.*

Clinical jaundice is usually first detected by the yellow discoloration of the visible mucous membrane such as the conjunctiva and sclera of the eye ball and the gums. It is well-known, however, that this discoloration only appears when excessive amounts of bile pigments have been circulating through the tissues for some time, while progressive absorption of pigment takes place. In tissues richly supplied by blood even a fairly severe degree of jaundice may be masked by the haemoglobin. In order therefore to detect a jaundice in the early stages, or alternatively to ascertain the efficacy with which the liver is excreting bile, it is essential to examine the blood serum for the presence of these pigments. This procedure has been adopted throughout. Experimental animals were regularly bled from the jugular vein and 10 c.c. citrated blood centrifuged at high speed. The supernatant plasma was drawn off into separate test tubes of uniform bore. Thereafter the colour was described as also its depth, and the v. d. Bergh reaction carried out where necessary. In all cases where the plasma was water clear or practically so, and the v. d. Bergh reaction negative, this was regarded as indicating a free and efficient bile excretion from the liver.

In order to test out the above point, 126 clinically healthy Merino sheep (ranging from 2-4 tooth in age) were bled and the plasma examined. The following results were obtained:—

20 water clear and v. d. Bergh negative.
100 very slight yellowish trace, v. d. Bergh negative.
6 definitely yellow—direct v. d. Bergh positive.

This indicates that by far the greatest majority of these animals show a clear or practically clear blood serum free from bile pigments while a small minority may be affected by a sluggish bile excretion as shown by the colour of the plasma and the presence of pigments. The healthy Merino sheep may thus be looked upon as an animal with a very efficient bile excretion and in which a physiological bilirubinaemia is not the rule. Regular examination of the blood serum therefore serves as an index of the bile flow.

2. *Bile Flow as recorded from Biliary Fistulae.*

In article 6 of this series the results of experimental ligation of the extra-hepatic bile tracts in sheep were recorded. In order to gain a more precise insight into the amounts of bile excreted over given periods, a further series of experiments were undertaken. For this purpose young Merino sheep (2-4 tooth old) and in good condition were selected. After a preliminary starvation of 24-36 hours a laparatomy was performed in the right flank under general anaesthesia using chloral hydrate intravenously. The common bile duct was then double ligated with thin silk and severed between the ligatures. An 8-inch length of fairly stiff rubber tubing (bore 4 m.m.) into one end of which a short funnel shaped glass tube had been inserted, was used as a cannula for the gall bladder. A small incision was made through the free end of the bladder and the flanged end of the tube tied into the bladder by means of a purse string suture, the other end of the tube being withdrawn to the exterior through a stab wound close to the laparatomy. Due to the fairly long gall bladder in the sheep it could as a rule be drawn up close to the stab wound. The laparatomy wound was subsequently closed with the usual three layers of sutures. The only dressing used was a thin layer of collodion-iodoform over the injured skin. The bile was collected in a flat thick-walled glass bottle strapped on to the side of the animal, the fistula tube penetrating a tight fitting cork stopper on the bottle. Sheep were usually operated on in pairs, and in some instances where liver bile was studied, the cannula was inserted into the neck of the gall bladder close up to the cystic duct while the gall bladder itself was removed. All animals were kept on a ration of veld hay, green lucerne and crushed yellow maize with water ad lib. Apart from the daily recording of the bile flow, the animals were bled each morning and the serum examined for pigments, as also the conjunctiva for clinical jaundice. All the faeces was collected in linen bags strapped on to the animals and weighed at 12 hourly intervals i.e. at 6 a.m. and 6 p.m. at the same time that the bile volume was recorded. Furthermore the animals were weighed twice weekly. Rectal temperatures were taken in the morning and in the evening.

The following records were obtained from the different sheep placed in experiments:—

Two Merino hamels (No. 1 and No. 2) were kept under observation for 6 days prior to operation. During this period both animals were feeding very well. In both sheep samples of blood withdrawn on several occasions showed the serum to be water clear and free from bile pigments. The animals were fed during the day only, all food being withdrawn from them at night. The following table indicates the weight of faeces collected over this period.

Day.	Sheep No. 1 (2 tooth hamel). Weight 57·5 lbs.	Sheep No. 2 (2 tooth hamel). Weight 50·5 lbs.
1. Day	204 grams	171 grams
Night	107 ,,	10 ,,
2. Day	113 grams	99 grams
Night	77 ,,	55 ,,
3. Day	116 grams	92 grams
Night	24 ,,	96 ,,
4. Day	250 grams	178 grams
Night	132 ,,	155 ,,
5. Day	240 grams	168 grams
Night	154 ,,	169 ,,
6. Day	129 grams	130 grams
Night	106 ,,	117 ,,

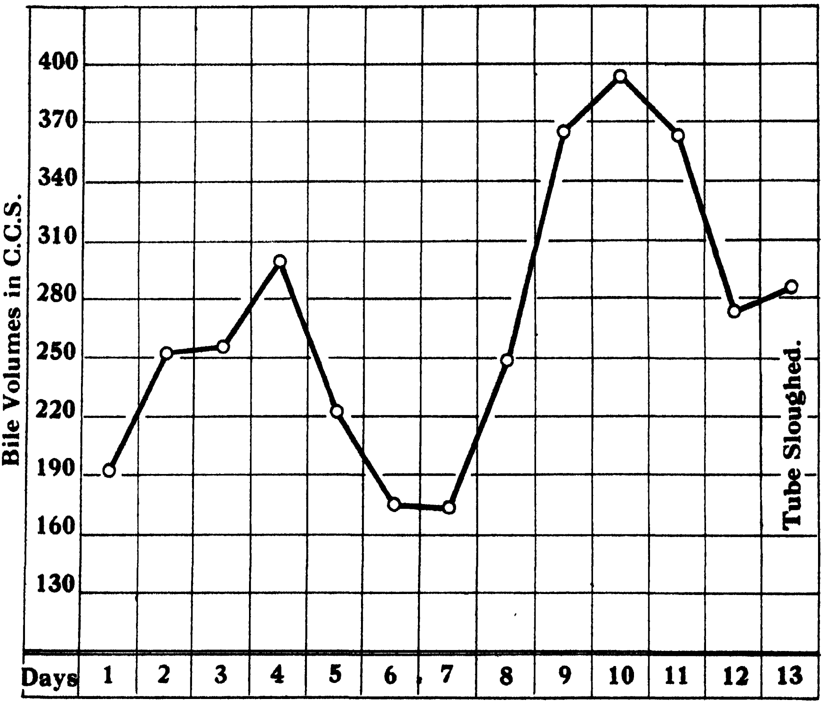
It is thus seen that on an average sheep No. 1 excreted 275 grams faeces daily of which 175 grams was passed out during the day and 100 grams during the night. For sheep No. 2 the corresponding figures were 240 grams daily, i.e. 140 grams by day and 100 grams by night. Regular examination of the faeces showed that in both sheep it consisted of well-formed glistening dark green pellets.

On the seventh day both sheep were operated and a rubber cannula inserted into the gall bladder after ligation of the common bile duct. Again the faeces were collected as well as all the bile excreted. For the rest the treatment remained as before . As will be noticed from the accompanying graphs (No. 1 and No. 2) the volume of bile eliminated daily showed fairly wide fluctuations, although it was flowing freely from the tube. Furtherfore there was no constant relationship between the " day " yield and the " night " yield. Thus on some days the former was slightly greater and vice versa, although on the whole it was found that approximately equal quantities were eliminated by day and by night. Consequently there was no suggestion of a constant diurnal rythm in the bile excreting activity of the liver. The average 24 hourly excretion in the two sheep was however remarkably close. Thus for sheep No. 1 this was 270 c.c. as compared with 265 c.c. for sheep No. 2.

The Bile.—Daily examination of the bile showed it to be of a very dark green colour, although on some days the shade was lighter. Usually it was clear and transparent, although again at times varying degrees of turbidity were noticeable. The viscosity throughout was very low due in all probability to little secretion of mucus from the bile tracts. Moreover as the bile was being rapidly drained from the gall bladder little opportunity was given for water absorption and hence for a concentration of the bile.

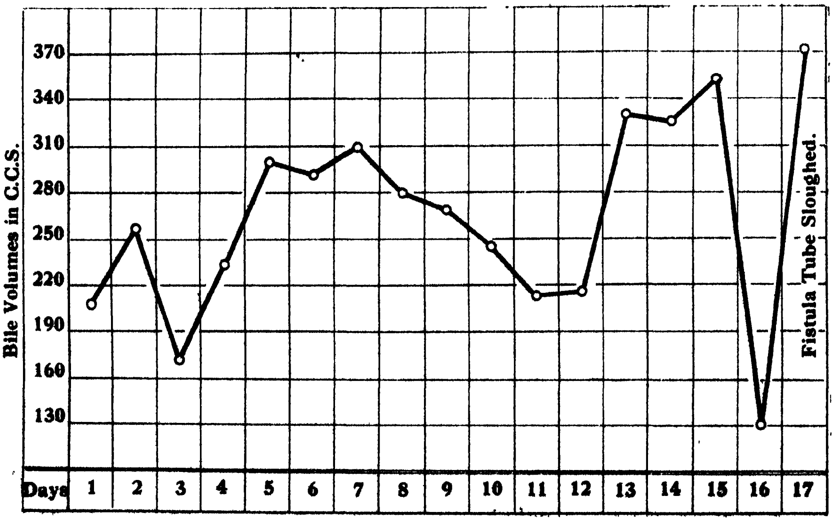
GRAPH No. 1.

Bile Volumes of Sheep No. 1.



GRAPH No. 2.

Bile Volumes of Sheep No. 2.



The Serum.—On the whole the blood serum remained water clear, although on some days a very slight yellowish coloration was noticeable and giving a doubtful direct van den Bergh reaction. From this one is led to conclude that the bile excretion from the liver, except for an occasional slight sluggishness, remains very efficient in spite of the fact that no bile enters the intestines and thus rendered incapable of exerting any cholagogue action. In sheep No. 1 the bile cannula sloughed away on the 15th day after operation leaving a very small constricted skin wound through which practically no bile was passing. This was followed by a rapid increase in bile pigments in the serum, which in 48 hours was of a deep yellow colour. At this moment too the animal developed acute photosensitisation with swellings of the lips and ears due to phylloerythrin present in the bile and regurgitating with it into the general circulation. After 4 days the photosensitisation passed off while the serum gradually lost the yellow coloration and again became water clear after 8 days, in spite of the fact that the fistula wound was completely closed by this time. When the animal was killed for post-mortem examination 4 months afterwards, restitution of the common bile duct was found to have taken place with bile entering the duodenum in the usual way.

In sheep No. 2 the tube sloughed on the 19th day after operation with bile continuing to flow from the skin fistula. On the 26th day the animal was killed for post-mortem on account of its poor condition.

Faeces.—Up to the time of the sloughing of the tubes the appetite of both animals remained good. The average daily yield of faeces for Sheep No. 1 amounted to 170 grams and for sheep No. 2 it was 95 grams. In both cases the consistence of the faeces showed little change from the normally well formed pellets. The colour however changed from the usual greenish black to a lighter greyish brown following the prevention of bile from entering the intestines.

Once it was established that by inserting a rubber cannula into the gall bladder of a sheep, its total daily yield of bile could be caught up and accurately measured, further experiments were undertaken with the object of ascertaining how the bile flow could be influenced by various factors, i.e. either inhibited or stimulated

Factors causing Inhibition of Bile Flow.

As indicated previously (article 5) sheep dosed with the plant Lippia Rehmanni (Pears) develop an acute photosensitisation accompanied by jaundice, both symptoms closely resembling those seen in true geeldikkop. In order therefore to gain further insight into the genesis of icterus especially of the obstructive or regurgitative type, i.e. of the factors which inhibit the prompt excretion of bile from the liver, an extensive series of experiments were undertaken with the plant Lippia Rehmanni. As will be indicated later, various other substances including some well-known liver poisons were also tested out on sheep although in no case was the typical jaundice of geeldikkop or Lippia poisoning produced.

The Lippia material consisted of large quantities of mature plants collected during the summer months. This was dried in the shade, then very finely pulverised in a mill and stored in stoppered jars. Experimental animals were drenched by stomach tube either with watery suspensions of the plants or 96 per cent. alcoholic extracts after evaporation of the alcohol.

1. *Drenching of Alcoholic Extracts of Lippia to Normal Sheep.*

Seven young Merino hamels (2 tooth) were each dosed with alcoholic extract from 500 grams Lippia and killed at regular intervals for post-mortem examination and collection of materials. In each case the serum was definitely yellow within 24 hours while after 48 hours it had changed to a deep brownish yellow and giving a strong direct van den Bergh reaction. On the third day all these animals showed a light but definite clinical icterus on the sclera and conjunctiva. Furthermore at this stage they all became acutely photosensitive with swelling of the head and accompanied by intense skin irritation. No haemolytic processes could be detected as daily determinations of the percentage volume of red cells remained constant and the serum free from haemoglobin.

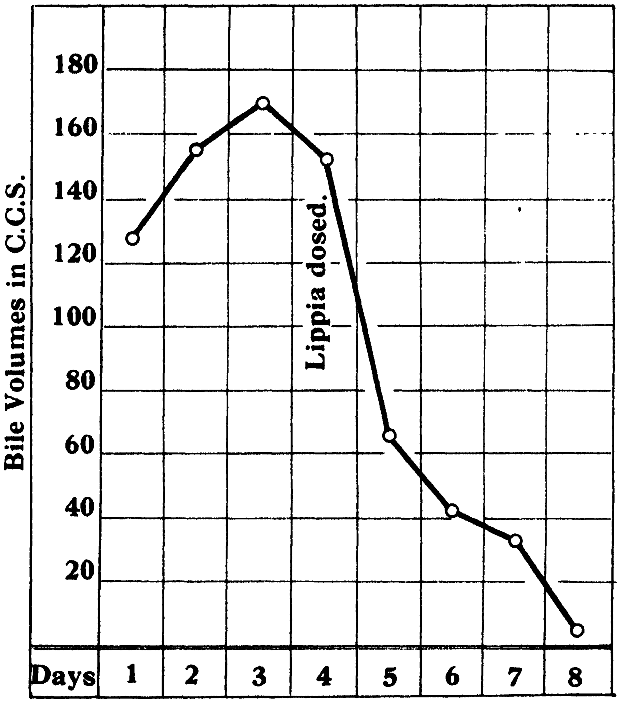
2. *Drenching of Lippia to Sheep with Biliary Fistula.*

As Lippia causes such a prompt bilirubinaemia, it was hoped that its quantitative effect on the bile flow would be more clearly shown after dosing it to animals with a biliary fistula. The following graph illustrates the effect of 500 grams Lippia dosed to a fistula sheep, a similar reaction being noted in several sheep treated in this way (Graph 3). Thus it is seen that an average daily bile flow of 150 c.c. was reduced to 66 c.c. within 24 hours after dosing Lippia, while by the 4th day, the flow was practically completely stopped, whereas the jaundice rapidly became more pronounced. Furthermore the colour of the bile undergoes a marked change after dosing the animal with Lippia. Thus in an animal showing a continuous flow of dark greenish bile from the fistula, when dosed with the alcoholic extract from 400 grams Lippia, the colour changes from the dark green to a light yellowish green within 5 hours of dosing. After 24 hours it changes to a clear pale sea green, whereas 3 hours later the bile flow is practically colourless and water clear resembling the socalled "white bile" (see coloured plate). It is only after a period of 4-5 days when the liver is regaining its power of bile excretion that the colour of the bile also deepens, i.e. a renewed elimination of the pigments takes place. At the same time the serum clears up and a general improvement in the health of the animal becomes noticeable, since during the period of severe jaundice all animals appear markedly depressed with loss of appetite, decreased ruminal movements and a stasis of the large intestines resulting in constipation. In this respect all the findings appear to be identical with those described for true geeldikkop or those resulting from artificial obstruction to the bile flow through ligation of the bile tracts (Quin 1933).

Factors intended to cause Stimulation of Bile Excretion.

Seeing that the plant *Lippia Rehmanni* possesses such a strong paralysing effect on the bile excretion, various experiments were undertaken in an attempt to overcome the jaundice and to re-establish the bile flow. It should be mentioned that in no single case of this type of jaundice are the extrahepatic bile tracts occluded, e.g. there is no undue distension of any part of the tract. Furthermore on post-mortem, bile flows through easily into the duodenum even after gentle pressure on the gall bladder.

Graph No. 3.
Effect of Lippia on bile flow of sheep.



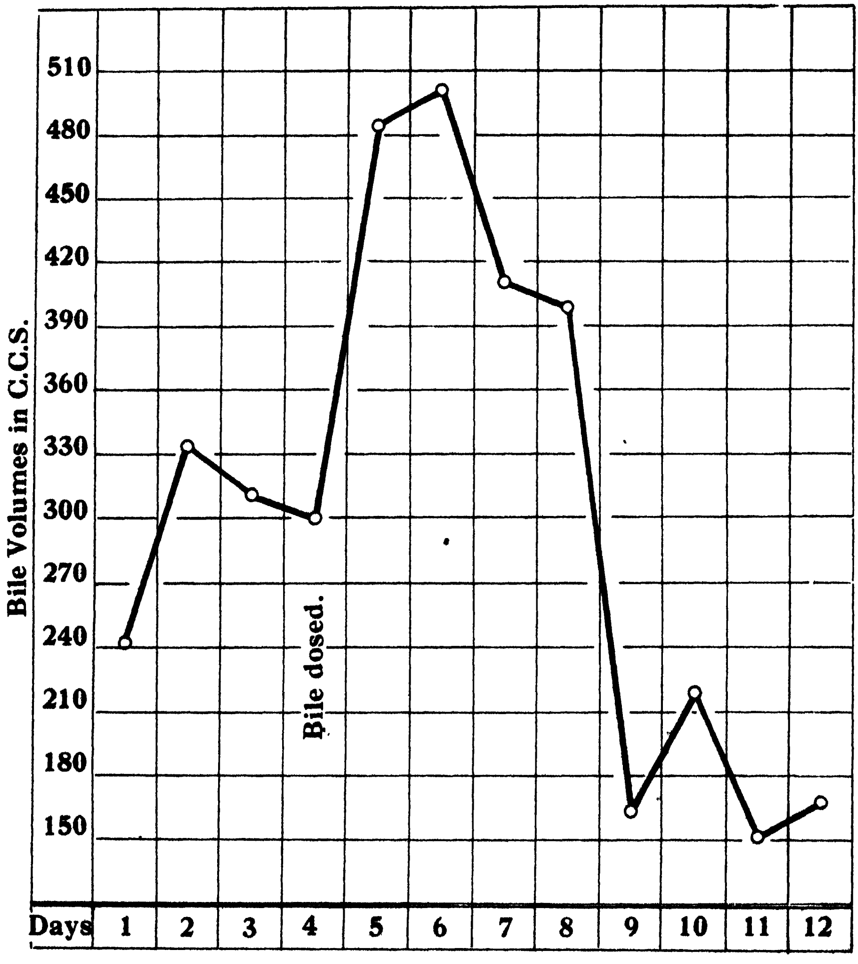
One is thus forced to conclude that some intrahepatic disturbance of the normal bile excretion is the primary effect caused by the Lippia. That being the case, it was reasonable to expect that the administration of certain materials might through their cholagogue action, stimulate the liver to renewed excretion. With this object in view, various materials were tested out, starting with the more commonly known cholagogues.

1. *Dosing of Bile to Sheep with a Bile Fistula.*

Seeing that bile through the action of its contained bile salts, is generally regarded as a particularly efficient cholagogue, large quantities were collected from sheep and also from other animals for dosing to experimental animals whose bile flow in turn, was closely followed over definite periods. Four sheep with a biliary fistula were dosed with sheeps bile 1,000 c.c. daily for 2 consecutive days. As will be seen from the accompanying graphs of the bile flow of two of these sheep (graphs 4 and 5) the average daily yield increased from 300 to 450 c.c. in the one case and in the other from an average of 220 c.c. to 366 c.c. per day over a period of 4 days. From these results it is clear that the dosing of large quantities of bile causes a transitory though definite increase in the bile flow of sheep with a biliary fistula.

GRAPH NO. 4.

Effect of dosing bile on the flow of bile in sheep with fistula.



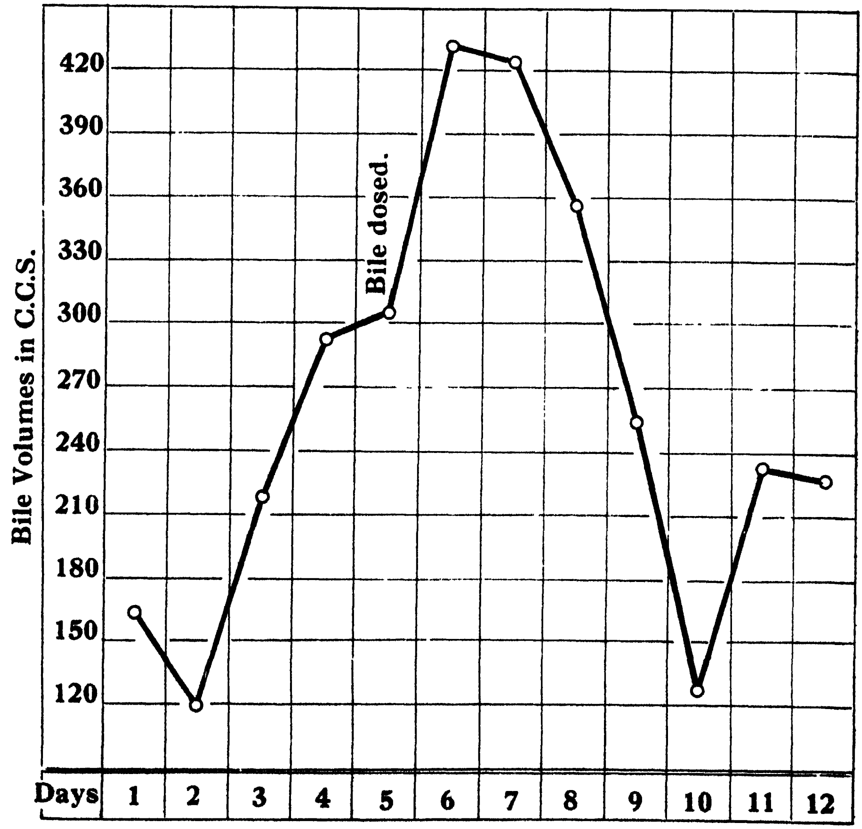
2. *The Cholagogue Action of Various Substances administered to Fistula Sheep after being Poisoned with Lippia.*

In these experiments the object was to ascertain the strength of various substances, some of which are reputed to posses definite bile driving powers, in overcoming the jaundice caused by the administration of Lippia. For this purpose 28 experimental sheep were used. Some of these carried bile fistulae and in which the daily bile flow could be recorded, while in other sheep daily comparisons of the serum colour served the purpose of ascertaining the degree of bilirubinaemia and hence the extent to which bile was being eliminated from the liver. The following materials were administered to different sheep suffering from the effects of Lippia poisoning, i.e. as soon as a definite bilirubinaemia had been established and the first sign of clinical jaundice had made its appearance. All animals received 500 grams Lippia as such or its equivalent amount of alcoholic extract dosed through a stomach tube:—

1. Bile dosed repeatedly in large quantities (1,000 c.c.).
2. Sodium taurocholate ·5 gram intravenously.
3. Calomel dosed in amounts of ·5 to 1 gram.
4. Magnesium sulphate per os in amounts of 50 grams to 70 grams.
5. Aloes 1-2 grams per os.
5. Castor oil 60 c.c.
7. Castor oil 60 c.c. + 5 drops croton oil.
8. Olive oil 120 c.c.
9. Hexamine and cholic acid 1 gram each per os.
10. Mercurochrome ·05 gram intravenously.

Graph No. 5.

Effect of dosing bile on flow of bile in sheep with fistula.



All the above-mentioned materials are usually considered to exert a purgative action and possibly also some degree of stimulation of the bile flow. In every case however the effect produced on the icterus of sheep suffering from Lippia poisoning was very disappointing. Although purging was eventually caused by the large doses of calomel, and magnesium sulphate, this only followed after repeated dosing. The jaundice however remained unaffected, the bile flow being scanty and the serum charged with large amounts of bile pigments.

In addition to the above, various other forms of treatment were tested out in an endeavour to stimulate bile flow after the onset of icterus, e.g. by dosing sodium salicylate, quinine sulphate, copper sulphate and subcutaneous injections of adrenalin without anv of these however producing any beneficial effect.

In another series of experiments, preventive treatment was undertaken against the jaundice. Thus, before and also after dosing Lippia, sheep were kept on an exclusive laxative feed of green barley and green lucerne for two weeks. This however had no influence on the severity of the jaundice. Similarly the regular daily dosing of 250 grams of glucose so as to increase the glycogen content of the liver had no effect on the jaundice. In order to prevent the Lippia from acting on the liver, attempts were then made to inactivate the toxic principle while passing through the digestive tract. Thus two sheep were dosed daily with 5 grams sulphur for 14 days before dosing the Lippia. In both these cases however the jaundice that followed was particularly severe. Three sheep which were dosed with 500 grams Lippia and immediately afterwards with 4 grams potassium permangenate in water remained healthy without any signs of photosensitisation or jaundice developing afterwards. When however in three other sheep dosed with Lippia, the potassium permanganate was given 24 hours later, it had no effect in preventing the onset of jaundice which was of the usual severity. This indicates that potassium permanganate most probably through its oxidative action, can inactivate the Lippia toxic principle before its absorption from the intestinal canal.

Experiments with Other Liver Poisons.

Of the various substances known to exert a poisonous effect on the liver, chloroform, carbon tetrachloride, phosphorus, and manganese chloride are amongst the most well-known ones. Experiments on Merino sheep were undertaken with each of these materials especially with the view of ascertaining to what extent they might influence the bile flow and thus lead to symptoms of jaundice.

Chloroform.—A full-grown sheep dosed by stomach tube with 8 c.c. chloroform in olive oil died within 24 hours. The lesions found were those of acute pulmonary oedema and congestion accompanied by severe fatty changes of the liver and kidneys, without however any signs of clinical jaundice. This indicated that chloroform was highly toxic for sheep thus necessitating the administration of smaller amounts in subsequent experiments. Thus four sheep dosed with amounts from ·5 c.c. to 2 c.c. daily for 5 days showed no untoward effect. Regular examination of the serum however revealed a light yellow colour which in spite of continued dosing, did not reach the stage of a definite clinical icterus. Neither was photosensitisation noticeable in any of these sheep. In one case the dosing was continued for 43 days starting with daily doses of 0·5 c.c. and increasing up to 8 c.c. daily until a total amount of 110 c.c. had been administered. After the second dose of 8 c.c. the animal died suddenly with symptoms of acute respiratory distress. The only lesions that could be found were those of pulmonary oedema and fatty changes of the liver. Although bilirubinaemia was repeatedly observed during the period of dosing, no clinical jaundice could be established, neither was the carcass icteric. Given subcutaneously chlorofrom is far more toxic as amounts of 2 c.c. have been found to cause death within 24 hours with the same lesions as described above.

Phosphorus.—This severe liver poison was administered to experimental sheep either subcutaneously as phosphorated cod liver oil in 1 c.c. doses or as ordinary yellow phosphorus ·01 gram dissolved in olive oil and dosed by stomach tube. One sheep dosed with a total of 14 c.c. phosphorated oil over a period of 8 days showed a definitely yellow serum on the seventh day with a direct van den Bergh reaction (35 mgs. per litre). Clinical jaundice however was slight. The animal died on the ninth day with severe fatty changes of the liver and catarrhal gastro-enteritis. Jaundice although present was very slight. One sheep injected with daily doses of 1 c.c. phosphorated oil for 5 days died on the tenth day with a pale yellow serum giving a faint direct van den Bergh reaction.

Altogether 5 sheep were poisoned with phosphorus. In every case very severe fatty changes of the liver were noted, the consistence being very soft and friable and the organ engorged with blood. General icterus if present was slight

Carbon Tetrachloride.—Two sheep were dosed daily over long periods as follows:—

(*a*) One sheep was dosed with a total amount of 524 c.c. carbon tetrachloride over a period of 42 days, starting with a daily dose of 4 c.c. This was gradually increased to 20 c.c. of which 13 consecutive doses were given before the animal died on the 43rd day. Every dose of carbon tetrachloride was mixed with an equal volume of alcohol (96 per cent) before dosing. Daily examination of the serum was carried out. After the third dose it turned a light yellow with a faintly positive direct van den Bergh reaction. The serum colour gradually increased to a deeper yellow up to a maximum of 29 mgs. bilirubin per litre.

(*b*) One sheep was dosed 304 c.c. carbon tetrachloride over a period of 35 days in doses as described above, the only difference being that the carbon tetrachloride in this case was dissolved in equal volumes of olive oil. The serum in this case also turned a deep yellow with a maximum reading of 44 mgs. bilirubin per litre. The animal died on the 36th day.

Both animals were kept on a ration of hay, green lucern, crushed maize and meat meal. The only clinical symptoms noticeable in these two animals during the experimental period consisted in a progressive loss in condition, frequent drowsiness, a slight and often indefinite jaundice with increasing pallor of the mucous membranes and a gradual drop in the red cell count.

On post-mortem examination, both carcasses showed considerable emaciation and a well marked anaemia, also pulmonary oedema and an extensive fatty degeneration of the liver and kidneys and atrophy of the ruminal wall. There was no sign of icterus in the carcasses.

Manganese Chloride.—Two sheep dosed with manganese chloride in doses of 5 gram daily and gradually increased to 15 grams died on the 12th day after a total amount of 112 grams had been dosed. In both cases the serum turned a pale yellow without however clinical jaundice becoming visible. On post-mortem examination severe liver degeneration was the main lesion noticeable.

DISCUSSION.

In the various experiments outlined above, attempts have been made to elucidate some of the important factors influencing the normal bile excretion in Merino sheep. This work was undertaken primarily with the view of explaining the severe generalised icterus seen in the disease geeldikkop caused by excessive ingestion of wilted Tribulus plants. In this disease the icterus is of an exceptionally intense paralytic nature in which the liver appears to lose all power of bile excretion, with the result that the bile is regurgitated into the blood and lymph stream. Except for the bile pigmentation there is, however, little morphological change of the liver parenchyme to indicate the type of damage caused. The effect, therefore, seems to consist very largely of a functional derangement of the liver. In previous articles of this series, it was pointed out that administration of the plant Lippia although in no way related to Tribulus, provoked a closely similar or identical symptom complex in experimental sheep. Moreover the symptoms following surgical obstruction to the bile flow appeared to be the same. The only difference between the latter condition and that caused either by Lippia or Tribulus is the marked cavernous like dilatations of the extra and intrahepatic bile tracts above the point of obstruction following ligation of the common bile duct. In Lippia and Tribulus poisoning it would appear that the liver makes no such attempt at accommodating the bile in its own channels.

In the sheep there is no physiological bilirubinaemia comparable to that found in some other species of domesticated animals. Judging from the clear appearance of the serum and the absence of pigments from it one can conclude that bile elimination is very efficient and that the liver treats the bile pigments as non-threshold bodies. In spite of this efficiency, however, the bile flow can be very severely depressed or totally inhibited in cases of Tribulosis or Lippia poisoning thus leading to a pronounced degree of icterus. From the results obtained from sheep with a biliary fistula it is seen that the daily bile flow amounts to volumes of over 200 c.c. When, therefore, elimination is interfered with, the severity of the jaundice can be well understood. The kidneys under these conditions compensate to some degree for the loss of the liver function as shown by the intensely yellow brown urine voided. The compensation however is not complete since the jaundice persists as long as liver action remains disturbed.

Investigations carried out on the effect of cholagogues on the bile flow of these experimental animals show that the dosing of bile definitely causes an increase in the bile flow from the liver. Bile elimination may however proceed at a steady rate in the absence of any bile constituents returning to the liver, i.e. the entero-hepatic bile salt circulation is not essential for the continued excretion of bile in the sheep. Moreover the appetite and digestion of such fistula animals are fairly well maintained although a slow and progressive decrease in the body weight is frequently noticeable, this being more evident in some animals than in others.

Under the influence of Lippia poisoning, the bile flow of experimental sheep can be very promptly depressed and ultimately even completely inhibited. The main effect of the Lippia toxin on the liver is as yet not clearly understood. It appears to have a paralysing effect on the normal bile excretion for although the liver cells themselves are still capable of allowing the bile to pass through as indicated by the direct van den Bergh reaction, the bile tracts including the smallest bile capillaries seem to be incapable of moving the bile in the normal manner and at the normal rate towards the large bile tracts. This sluggishness in the bile flow results in some of the biliary constituents, e.g. the pigments to escape into the small blood and lymph vessels and so carried back into the general circulation where an extensive and severe jaundice may be caused. The difficulty in explaining the genesis of the icterus arises from the fact that morphological changes in the liver may be slight even in very severe cases of jaundice. This point has also been stressed by other workers. Thus Cantarow and Stewart, studying the morphological changes in the liver and bile passages of cats with total biliary obstruction, state that " these observations seem to indicate that there is no demonstrable correlation in individual instances, between the changes in the liver and bile ducts and the serum bilirubin concentration at any given time during the period of total bile stasis ".

The various experiments undertaken with the object of re-establishing the normal bile flow after the onset of Lippia jaundice, have been disappointing in that none of the cholagogues or purgatives used had any definite beneficial effect on the icterus and on the bile flow. Improvement, when it did come about, appeared as a slow process and governed by the powers of the body itself.

Other liver poisons, such as chloroform and phosphorus which were also studied, caused acute and severe fatty changes of the liver and frequently accompanied by bilirubinaemia. In no case however was the same intense and persistent jaundice of " geeldikkop " or Lippia poisoning observed.

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Section IX.

Miscellaneous.

Curson, H. H. ... ... Anatomical Studies, No. 61. A comparison of two of the so-called Zebus 371

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Anatomical Studies, No. 61.

A Comparison of two of the so-called Zebus.

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INTRODUCTION.

AN idea of the uncertainty of the definition of the word " Zebu " may be formed by perusal of the *Encyclopaedia Britannica*, 14th Edition, 1929. Under " Zebu " (Vol. XXIII, p. 938) one reads: " Zebu (*Bos indicus*), an Indian species of ox, characterised by its light colour and the possession of a hump on the back. The sacred bulls of India belong to this species, which is much used for draught and farm work, and also supplies milk ".

On turning, however, to " cattle " (Vol. V, p. 46), it is learned, in a description furnished by Professor J. A. S. Watson, of Oxford, that cattle may be divided into six groups, one of which(¹) is the Zebu or " Eastern and African domesticated cattle "! Professor Watson, in referring to African domesticated cattle, presumably means those cattle resembling the Eastern type, *viz.*, the Shorthorned Zebu, and therefore apparently does not include the humped Sanga and Lateral-horned Zebu (Afrikander) types. If this view is correct, then no provision is made in his classification for Afrikander or Sanga cattle. If, however, one accepts that he means all African domesticated (obviously native) cattle, then he places in a single group, cattle that differ as widely as the Shorthorned Zebu, Lateral-horned Zebu, Sanga and Brachyceros.

The first definition given above resembles that of Lydekker (1912) who applies the designation " Zebu " to " the Indian humped cattle " (p. 147). Watson's definition is somewhat similar but not so precise as that of Kronacher (1921) who refers to the Zebu as " Buckelrinder ". In regard to the African Zebu, however, he (Kronacher) gives the synonym " Sanga " which further complicates matters, especially as Epstein uses the term Sanga for a group which is distinct from the Shorthorned Zebu.

Duerst (1931) simply gives " Hockerrind " as the equivalent of Zebu (p. 759), which agrees with Kronacher.

(¹) The other groups are:—(*a*) Buffalo, (*b*) Bison, (*c*) Yak, (*d*) Gaur, Gayal and Bantin and (*e*) Western or European domesticated cattle.

As indicated by Curson and Epstein (1934), the original Zebu would appear to have been the Lateral-horned beast (represented by the Afrikander to-day), which later with Brachyceros gave rise to the Shorthorned Zebu. In West Africa there is further a Lyre-horned Zebu also with a thoracic hump, but the skull is apparently short and broad, due to Hamitic influence.

Summing up, it would seem that one school refers to only Eastern cattle as Zebu, while the second includes all humped cattle whether Asiatic or African.

Provisionally it is intended to include under the term " Zebu " any humped bovine whether of Asiatic or African origin and the subjoined classification is suggested:--

- ZEBU
  - TRUE ZEBU (Asiatic origin).
    - 1. Original Lateral-horned zebu, now called *Afrikander*.
    - 2. *Shorthorned Zebu*, essentially of Asia but well represented in East Africa.
  - PSEUDO-ZEBU (African origin).
    - 3. *Sanga*.—Essentially African and well represented in South Africa.
    - 4. *Lyre horned Zebu*.—Best represented in West Africa.

The problem of classification is not simple in that either the skull or the hump may be taken as the determining factor. Above, the skull is the main factor, being generally relatively narrow in (1) and (2), but comparatively broad in (3) and (4).

The two Zebu groups discussed in this paper are the Lateral-horned and Shorthorned Zebus.

According to Epstein, Lateral-horned Zebus entered Africa about the end of the third pre-Christian millenium and during the New Kingdom of Egypt (1550-945 B.C., Yahuda) they were represented on mural decorations. Shorthorn Zebus on the other hand have been introduced into East Africa only during Christian times. The former are scattered throughout the Subcontinent whereas the latter occur along the East coast from Erithrea as far south as the Zambesi River.

In regard to South Africa, as far back as 1904 MacDonald, A. C., expressed himself as being " rather inclined to the idea that the Afrikander cattle are descended from one or other of the breeds which were brought down from North Africa by the native tribes ". On the other hand others have believed in an European origin, *e.g.* Holm (1912) supposes that " there exists an indirect though remote relationship between the Afrikander and the Devon ". During the past decade or so general opinion has supported MacDonald, and Bosman (1924), for example, writes, " It would seem therefore that the foundation of the Afrikander breed could be attributed to the Hottentot cattle that accompanied the Hottentots to the South on their migration along the West Coast ". It was, however, not until 1933 that definite evidence, *e.g.* historical and anatomical, was brought forward by Epstein that the Afrikander was the Zebu in its original form, and that it arose in Asia probably from *Bos namadicus*, whose fossilised remains have been described by Duerst (1908).

In 1934 Epstein discussed the physical characteristics and distribution of both the Lateral-horned Zebu and the Shorthorned Zebu; and in the same year with Curson briefly described the skulls of the three *parent* stocks of African cattle, *viz.* Hamitic Longhorn, Brachyceros and Lateral-horned Zebu (Afrikander). The skulls of typical members of the *derived* types, *viz.* the Shorthorned Zebu, Lyre-horned Zebu, Sanga or so-called Kaffir cattle (see footnote 4) have not yet received special attention.

Epstein (1934) believes that the Shorthorned Zebu encountered to-day in Asia, principally, India, and Africa reached Africa " in the post-Christian era, when the power of Persia was at its zenith and afterwards during the Arab invasion ". It is furthermore not a pure Zebu in that it represents the influence in Asia of Brachyceros (the first Asiatic bovine immigrant into Egypt) on the Lateral-horned Zebu. Thus the Shorthorned Zebu is a type derived through the intermingling in Asia of the original Brachyceros (still predominant along the littoral of West, North and North-East Africa) and the original Lateral-horned Zebu or Afrikander, as it is called in the Subcontinent to-day.

In India, although the Longhorned Zebu is well represented, *e.g.* in the Amrat Mahal cattle, not only is the horn *not* laterally disposed as in the Afrikander, but the hump is dissimilar in situation and structure. Gunn (1909) in describing the cattle of South India writes " Among the breeds found in Mysore the first place is undoubtedly due to the Amrat Mahal. The Amrat Mahal, literally Milk Department, is an establishment for the breeding of a race of cattle peculiar to the country of Mysore . . . and so distinctive is this breed that they may readily be distinguished from every other breed in India ". See Fig. 17.

That there are longhorned Zebus in India possessing *laterally* directed horns would appear to be the case from a photograph of the skull of a " Longhorned Zebu ox from Nepal, British Museum ". shown on p. 99 by Epstein in his M.S. on *The Red Afrikander Cattle.*

Lydekker (1912) gives the term " Zebu " as " the designation of the Indian humped cattle "(²); but as is clear from what has been said before, the original (Lateral-horned) and later (Shorthorned) Zebus vary in origin. As a result, there are anatomical differences as well as resemblances and it is these which will receive attention in this study.

As evidence of " the undoubted distinctness of humped cattle " (*Bos indicus*) from European cattle (*Bos taurus*), Lydekker mentions the characters indicated by the late Mr. Edward Blyth in the *Indian Field* for 1858. They are as follows:—

(*a*) " In general configuration ", *e.g.* head, dewlap and hump;
(*b*) " in the shape of the ears ";
(*c*) " in the point where the dewlap commences ";
(*d*) " in the typical curvature of their horns ";
(*e*) " in their manner of carrying their heads when at rest ";

(²) The Zebu received its name *Bos indicus* from Linnaeus in the XVIII century (Lydekker, p. 149).

(*f*) "in their ordinary variations of colour, especially in the frequent presence of nilgai-like markings on their feet "(³);

(*g*) "they have different habits and their voice is entirely different". Lydekker adds that it is "more of the nature of a grunt than a low";

(*h*) "humped cattle in India seldom seek shade and never go into the water and there stand knee-deep, like the cattle of Europe"; and

(*i*) "they have given rise to many distinct breeds, differing greatly in size, in the presence of either one or two humps, in length of horns, and in several other respects". Two humps is, of course, incorrect.

While Blyth's description is based on Indian cattle, Lydekker himself had in mind *also* African Sanga cattle, *e.g.* Galla cattle(⁴) of Abyssinia, Watusi cattle of East Africa and Ngami cattle of Bechuanaland. Strangely Lydekker describes Afrikanders as being "a breed of long-horned cattle *without* humps".

Epstein (1934) emphasised the value of some of the above criteria, *e.g.* (*b*), *e*), and (*g*), and adds speed and docility as distinctive features; but as will be shown later, the anatomical characteristics, particularly skull, dewlap, hump and bifid spines of the more caudal thoracic vertebrae, seem to be the most reliable guides.

POINTS OF RESEMBLANCE.

The anatomical features common to both Zebu types are:—

External.

(*a*) *Head.*—From frontal view, the head is generally long and narrow (coffin-shaped) and the orbital region is *not* so pronounced as, for example, in the Sanga. On lateral view, the profile is generally convex, the most prominent point being behind and above the eyes. Convexity is less marked in females.

(*b*) *Dewlap.*—"Well developed, tied in slightly at throat, starting from chin to back of chest "(⁵).

(³) Lydekker explains (p. 150) that these "take the form of white rings round the fetlocks in the darker coloured Indian strains or individuals". Kelley (1932) in comparing European and Indian cattle (p. 15) makes no reference to voice.

(⁴) Lydekker uses Sanga as a synonym of Galla, and Kronacher (1921) in the widest sense as including any humped African bovine. Epstein, however, considers that it represents the type originating from the Hamitic Longhorn and Lateral-horned Zebu intermixture.

(⁵) This is taken from the scale of points adopted in March, 1932, by the Afrikander Cattle Breeders Society. The meeting resulting in the foundation of the Society was held at Potchefstroom in June, 1912.

Bisschop (Lectures, Faculty of Veterinary Science, University of Pretoria) gives the following description: "The dewlap commences from the chin as two separate folds which converge a few inches further back. In the region of the throat the dewlap shows an indentation, but from this point backwards it hangs evenly and conspicuously to well between the front legs. In the region of the brisket it may be rather pendulous and so create the impression that the thorax is deeper than is actually the case. The dewlap is never "filled" but consists of two directly apposed layers of skin. Vertical folds of the dewlap such as sometimes seen in the Short-horned Zebu are considered undesirable."

(c) *Hump.*—Large and prominent.

Internal.

(i) *Skull: Frontal surface.*—Long and comparatively narrow. The margin of the orbit is not prominent, and the profile is convex. The stalk of the horn core is well marked.

Lateral surface.—The temporal fossa is deep and curved and the horn has a lateral direction.

Nuchal surface.—The frontal ridge is thick and prominent especially centrally. From the front it is convex and curved from side to side. As a result the fronto-nuchal angle is acute. It is apparent that the features of the head are governed by the form of the skull.

(ii) *Dorsal Vertebra.*—The superior spines are bifid from the sixth vertebra caudally. As will be seen from Figs. 11, 12 and 16, not only is the upper one-fifth divided medially, but it is also compressed antero-posteriorly.

POINTS OF DIFFERENCE.

Typical specimens of the two Zebu types are differentiated thus:

Feature.	*Lateral-horned Zebu (Afrikander).*	*Shorthorned Zebu* (⁶).
External.		
(1) Horn—		
(a) Length......	Long and slender............	Comparatively short.
(b) Direction....	Lateral......................	Upward and lateral.
(2) Hump—		
(a) Situation....	Cervico-thoracic.............	Thoracic.
(b) Attachment..	Firm........................	Less firm.
(c) Shape.......	Less prominent and generally pyramidal	More prominent and generally dome-shaped.
(3) Colour..........	Chiefly red..................	Of many colours.
(4) Size.............	Large beef type..............	Of all sizes, chiefly small.
Internal.		
(1) Temporal fossa...	Deep and markedly curved, being much influenced by lateral direction of horn core	Deep and less markedly curved owing to upward direction of horn core.
(2) Horn core—		
(a) *Direction*....	Lateral......................	Upward and lateral.
(b) *Base*........	Pearled wreath absent.........	African Zebus have frequently a pearled wreath (⁷).
(c) *Cross section.*	Oval........................	Circular.
(3) Hump—		
(a) Structure....	Muscular. See Fig. 13........	Musculo-fatty. See Fig. 14.

(⁶) Many of these features obviously depend on Brachyceros influence.

(⁷) It is possible this is a difference between typical Asiatic and African Shorthorned Zebus. This feature is derived from the Hamitic Longhorn, and is marked also in Sanga cattle.

SUMMARY.

Summarising the position(⁸) we have:—

Resemblances.

1. Both Zebu types originated in Asia.

2. *Externally*:—
   (*a*) They have a coffin-shaped head with convex profile;
   (*b*) the dewlap is prominent;
   (*c*) the hump is well marked, but not characteristic, for it occurs also in the Sanga type.

3. *Internally*:—
   (*a*) The thoracic vertebrae from No. 6 backwards have bifid spines.

4. Both have adapted themselves well to unfavourable environmental conditions, *e.g.* poor pasture, and parasites.

Differences.

Feature.	*Lateral-horned Zebu.*	*Shorthorned Zebu* (⁹).
1. Origin...............	A pure parent stock........	A derived type arising through intermixture of Lateralhorned Zebu and Brachyceros.
2. Arrival in Africa......	In pre-Christian times......	In Christian times.
3. Distribution in Africa.	Scattered and mainly south of the Zambesi River	Form compact groups chiefly in East Africa, north of the Zambesi River.
4. Horns................	Are oval in cross-section, long and laterally placed	Are circular at base, short to medium length and upright.
5. Hump...............	Cervico-thoracic and muscular	Thoracic and musculo-fatty.

ACKNOWLEDGEMENT.

It is a pleasure to express indebtedness not only to the workers with whom I am constantly in touch, *viz.* H. E. Hornby, Esq., F.R.C.V.S., Director of Veterinary Services, Tanganyika, Major H. H. Brassey-Edwards, M.R.C.V.S., C.V.O., Kenya, and Dr. H. Epstein, at present at the British Museum; but also to A. D. MacGregor, Esq., F.R.C.V.S., Principal, Bengal Veterinary College, Calcutta, and J. H. R. Bisschop, Esq., B.V.Sc., Onderstepoort, each of whom has provided me with important information.

Mr. Meyer's photographs are as usual excellent.

(⁸) A useful list of references concerning the Afrikander is given in an Appendix to Curson and Bisschop's paper (Anatomical Study No. 60) *Some Comments on the Hump of African Cattle. Onderstepoort Jl. Vet. Sc. Anim. Indus.* Vol. 5, No. 2. Oct., 1935.

(⁹) There are of course big differences between the various breeds of Shorthorned Zebus. Matson, quoted by Kelley (1932), divides the Indian Zebu into five chief subtypes.

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ADDENDUM.

Anatomical Study No. 61 was submitted to Dr. H. Epstein, who in a reply (10/4/36) to the Director of Veterinary Services kindly made the following observations:—

" I would like to pass a few comments on Dr. Curson's paper Anatomical Studies No. 61.

(*a*) On page 2 (the fifth line down) he writes with reference to the Brachyceros cattle: " The first Asiatic bovine immigrant into Egypt ". I no longer hold the view that the Hamitic Longhorn cattle were pure descendants of *Bos primigenius Hahni*, but rather that they originated from a mixture of Primigenius cattle imported into Africa from Asia by Hamitic immigrants, and the local Egyptian variety of *Bos primigenius*, viz., *Bos primigenius Hahni* Hilzh. Therefore the Brachyceros breed was the second bovine immigrant from Asia. But even if the Hamitic Longhorn cattle were pure descendants of *Bos primigenius Hahni*, their upright lyre-shaped horns would be no obstacle to such an assumption (as you suggested in the above mentioned letter). For it is one of the first effects of domestication that the heavy horn of the wild beast becomes lighter and consequently changes its position. Duerst (1926) gives an account of these changes in his work *Das Horn der Cavicornia.*

(b) Referring to the same paragraph in Anatomical Studies No. 61, I am still doubtful whether all shorthorned zebu breeds owe their short horns to the intermingling in Asia of the original Brachyceros with the original Longhorned Zebu(¹⁰). It is quite feasible that numerous shorthorned zebu breeds acquired their shorter horns during the long process of domestication spontaneously without any external " assistance ". (The difference in the structure of the hump between the Shorthorned Zebu and the Afrikander may also be attributable to the long period of domestication in Asia, and need not of necessity owe its origin to the influence of other cattle on the Longhorned Zebu.)

(*c*) *re* page 2 lower down:—The skull of the Longhorned Zebu ox from Nepal in the Kensington Branch of the British Museum does not possess laterally directed horns, but upright lyre-shaped ones. In all other respects, however, it resembles the Afrikander skull.

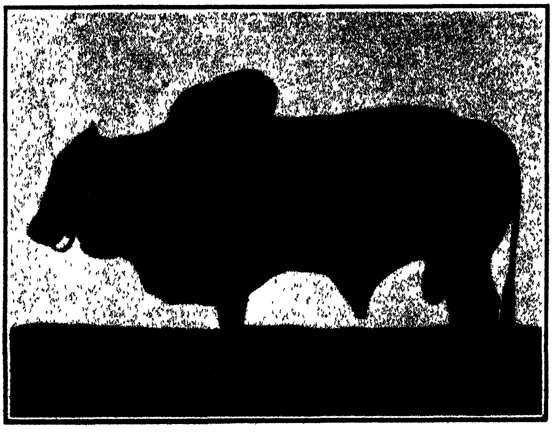
I am very grateful for the assistance and interest you have shown in my work which is always a great encouragement to me ".

(¹⁰) or Afrikander. Obviously one may consider the Shorthorned Zebu as constituting a parent stock, at any rate as far as Africa is concerned (see Curson and Epstein, 1934). *Footnote by H.H.C.*

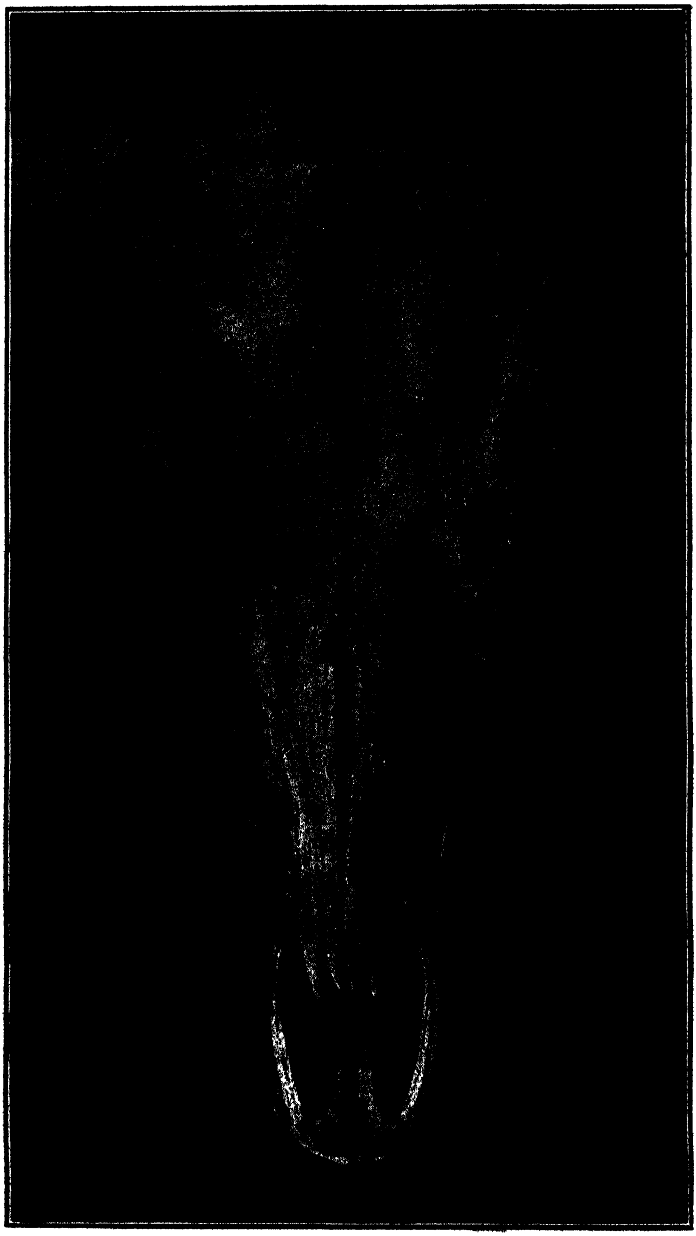
EXPLANATION OF FIGURES.

Figures 3-12 and 16 are approximately one-quarter of original.

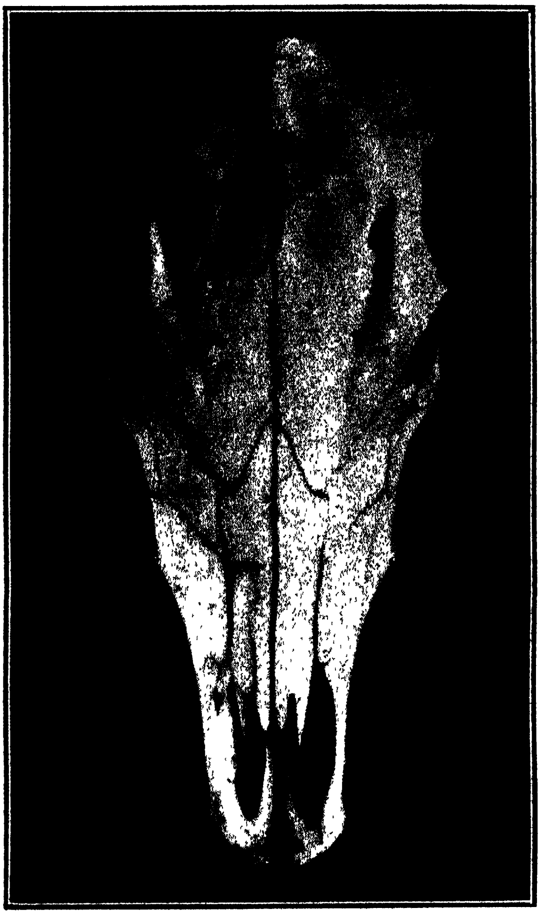
1. *Lateral-horned Zebu* (*Afrikander*) Bull " Holmesdale Stemreg ". Photo sent by former owner, Dodds Pringle, Esq., Jun., P.O. Adelaide, C.P., Colour light red, born 1917.



2. *Shorthorned Zebu* (*Tanganyika*) Bull (McCall, F. J., Ann. Rept. for 1929, Tanganyika). The Shorthorned Zebu from the East came in such large numbers (over a long period of time) that it eventually replaced the Sanga. There seems very little difference, if any, between the Asiatic and the African Shorthorned Zebu.

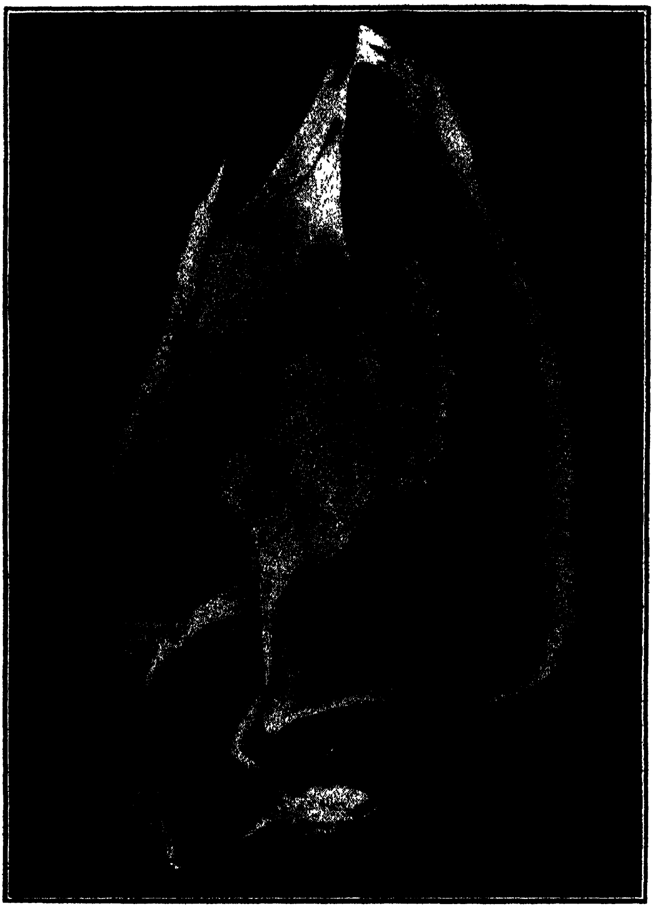


3. Skull of *Lateral-horned Zebu* (*Afrikander*) Ox A. 26, Onderstepoort. Front view. Length of "forehead" is 260 mm. Note lateral horn-cores with distinct neck, convex frontal ridge, and generally long coffin-shaped appearance of skull—Curson and Epstein, 1934.

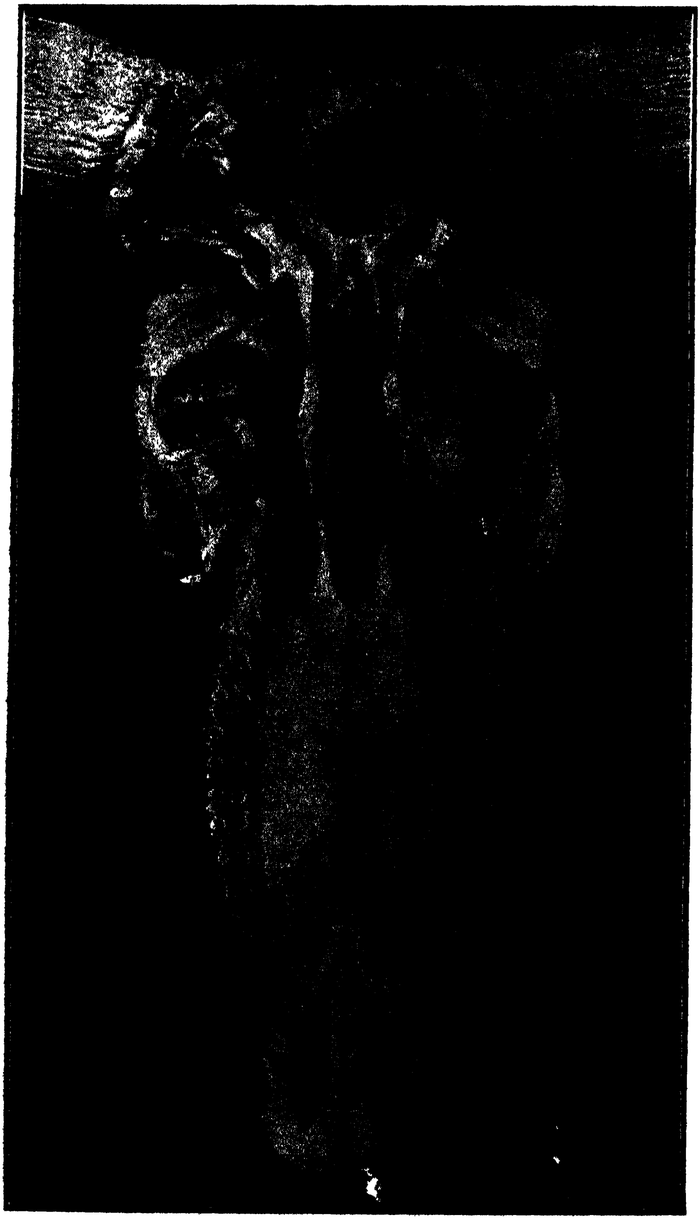


4. Front view of skull of *Shorthorned Zebu* (*Tanganyka-Masai*) Cow A. 20. Onderstepoort. Sent by McCall, F. J. Note the comparatively short horns and elongated head. Length of " forehead " is 201 mm.

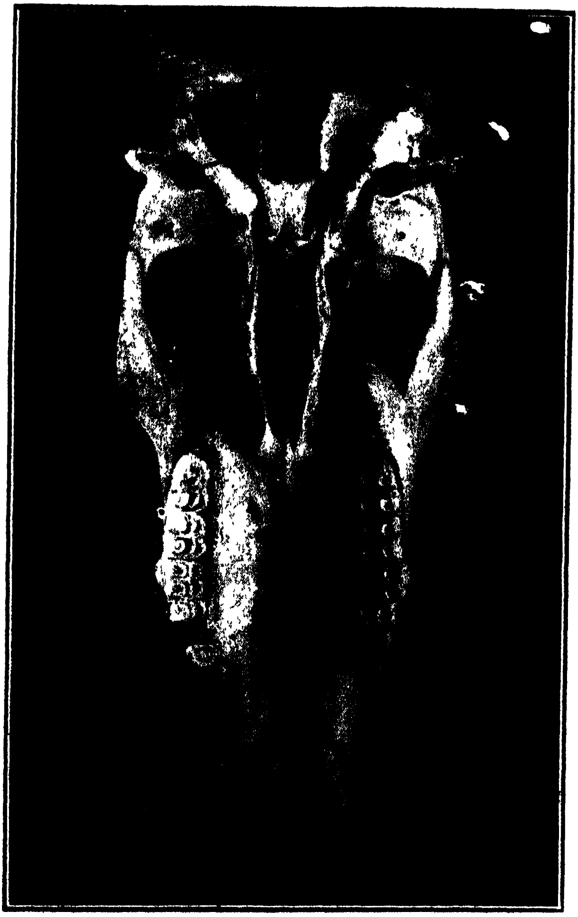
5. Lateral view of skull of *Lateral-horned Zebu (Afrikander)* Ox A. 26. Observe the strongly convex profile, especially over cranium and the deep and curved temporal fossa—Curson and Epstein, 1934.



6. Lateral view of skull of *Shorthorned Zebu (Tanganyika-Masai)* Cow A. 20. Onderstepoort.



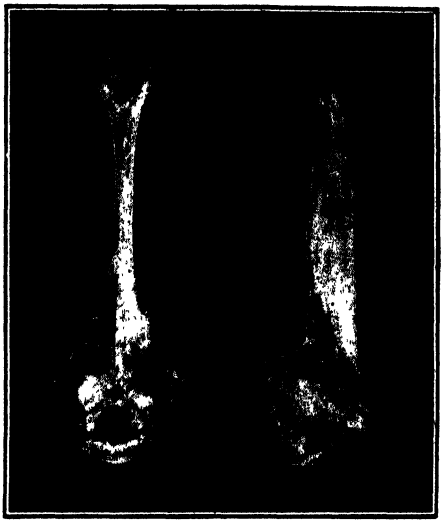
7. Nuchal view of skull of *Lateral-horned Zebu* (*Afrikander*) Ox A.26. Frontal ridge is prominent and convex from side to side. The median occipital crest is scarcely perceptible—Curson and Epstein, 1934.



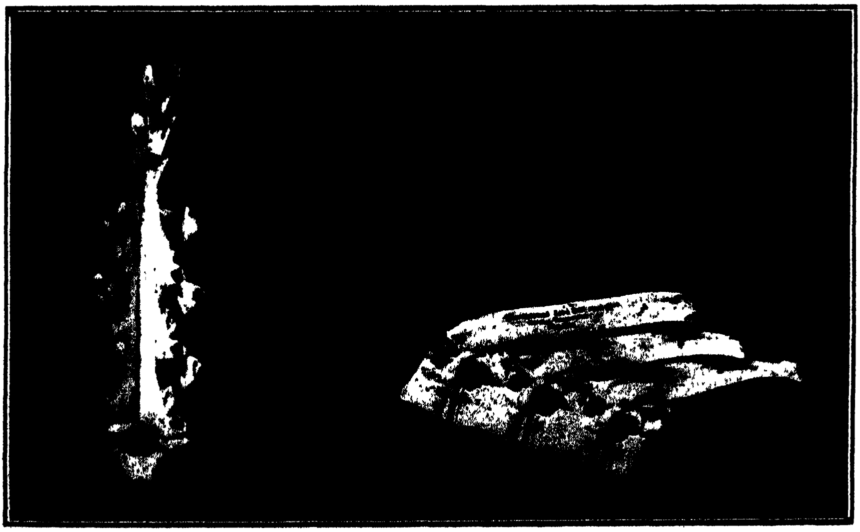
8. Nuchal view of skull of Cow A. 20.

9. Palatine view of skull of *Lateral-horned Zebu (Afrikander)* Ox A. 26 Long coffin-shaped appearance is marked, as is the neck at the base of the horn-core.

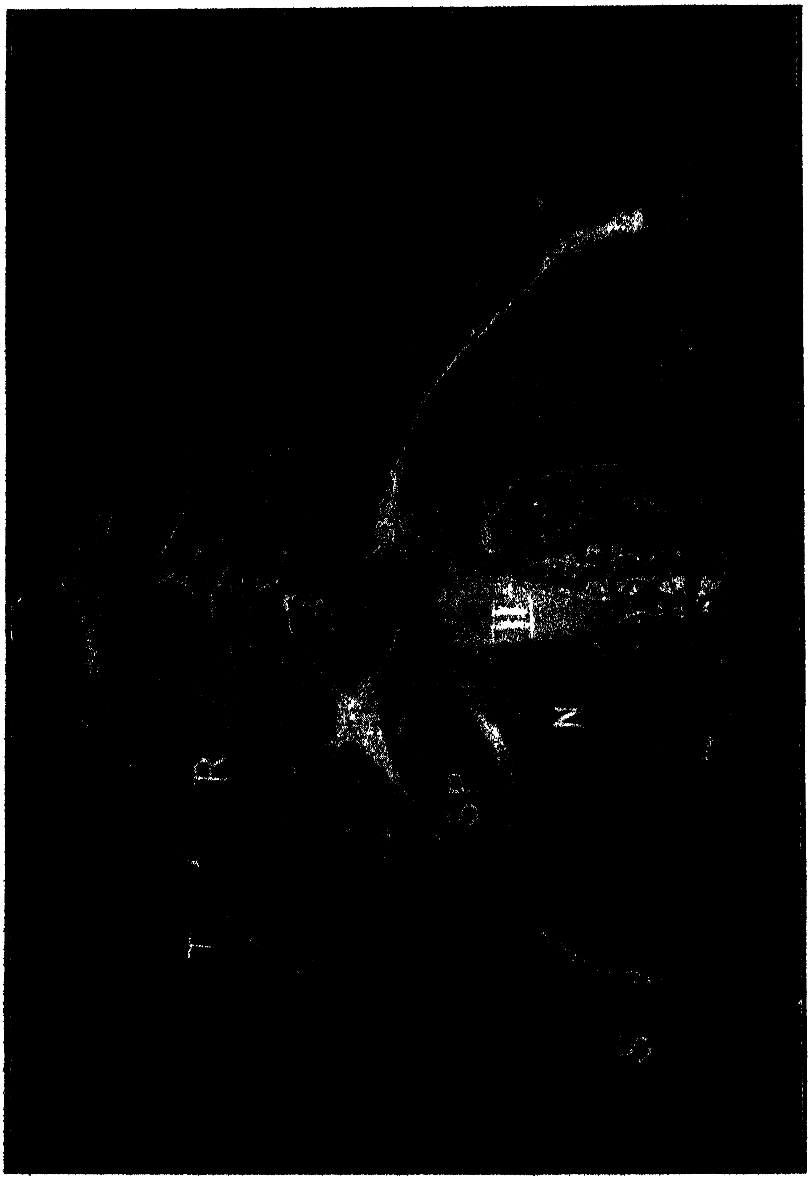
10. Palatine view of skull of Cow A. 20. Note the well definea neck at the base of the horn-core.



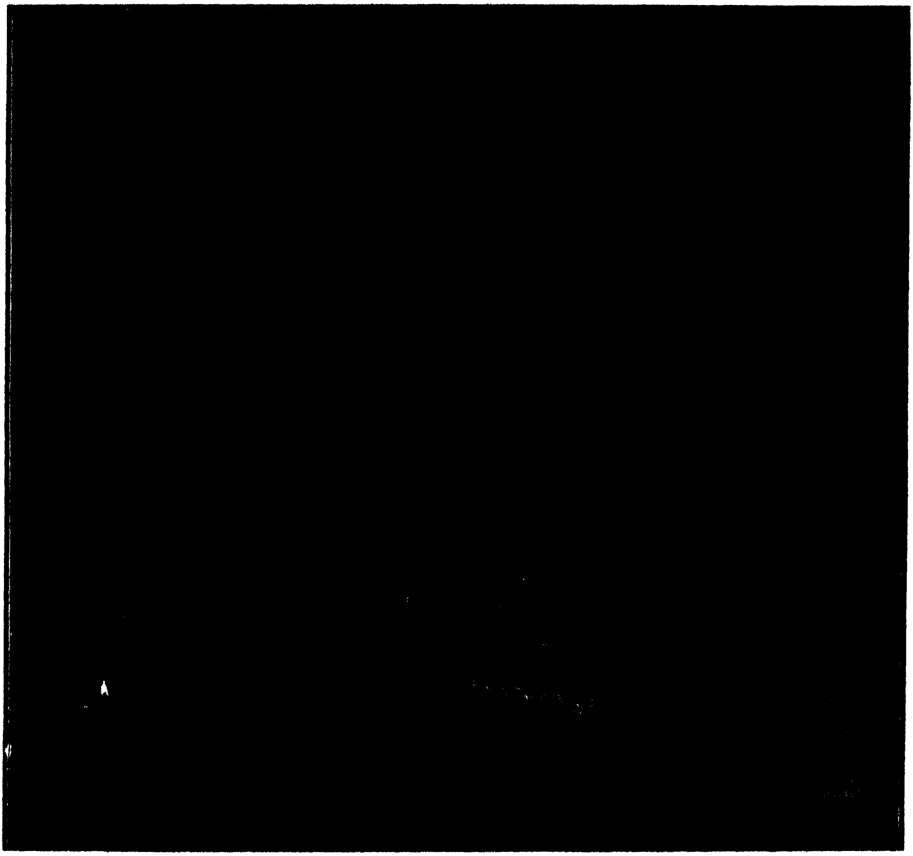
11. *Lateral-horned Zebu* (*Afrikander*) Cow (Curson). Front and lateral views of 9th dorsal vertebra. The superior dorsal spine is cleft—Curson and Epstein, 1934.



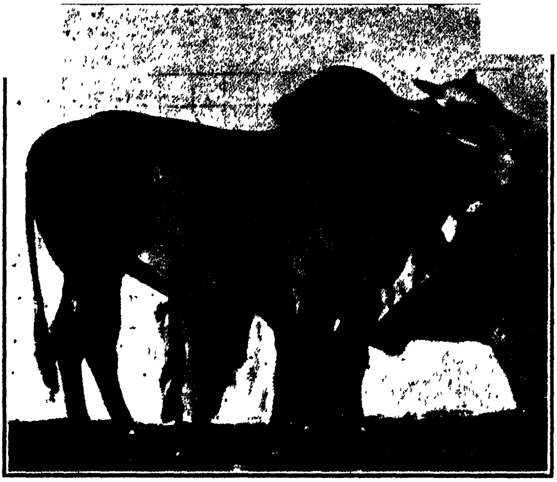
12. *Shorthorned Zebu* (*Tanganyika*) Bull. Front and lateral views of the 7th-9th dorsal vertebra (from H. E. Hornby, Esq., O.B.E., F.R.C.V.S., Mpapwa).



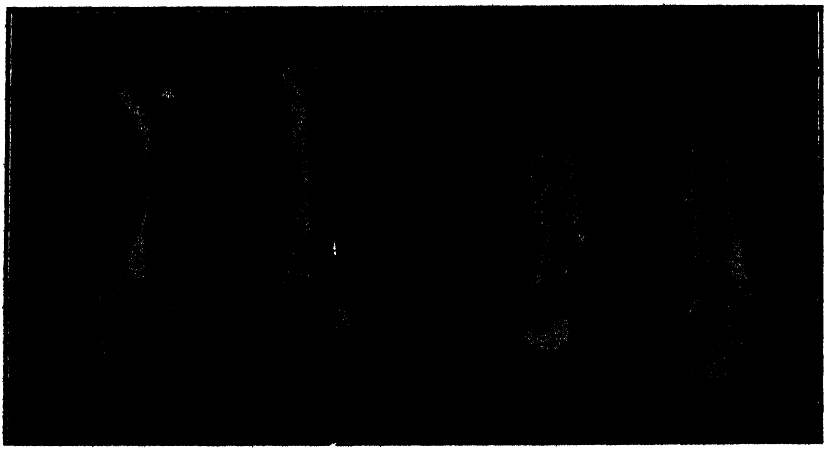
13. *Lateral-horned Zebu* (*Afrikander*) Bull 5572. Cross section of summit of hump over second thoracic vertebra, showing the following structure: *Mm. trapezius, rhomboideus, serratus ventralis and splenius, and ligamentum nuchae*—Curson and Bisschop, 1935—Magn. 2/3.



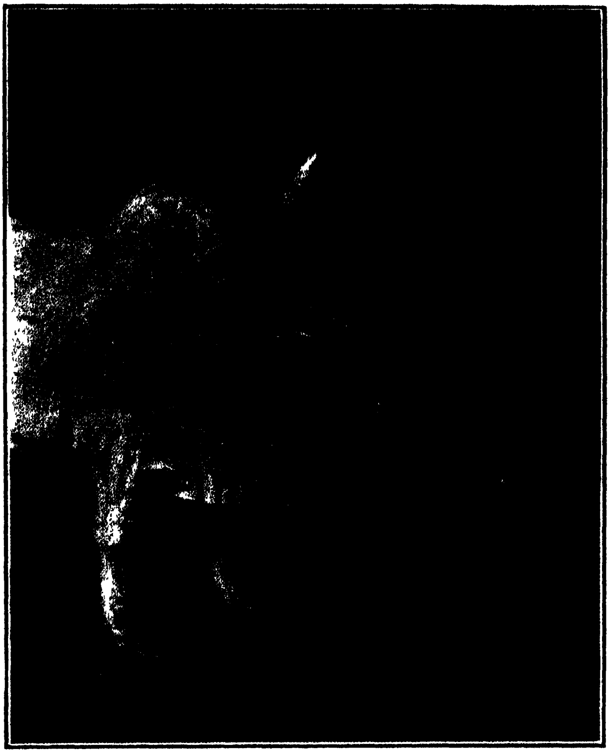
14. *Shorthorned Zebu* (*Tanganyika*) Calf (Hornby). Cross section of summit of hump over fourth thoracic vertebra. Distorted appearance due to tight packing in drum—Curson and Bisschop, 1935—Magn. 1¼.



15. *Shorthorned Zebu* (*Krishna Valley*) Bull. (McCall, F. J. *Annual Report* for 1929, Tanganyika.) Imported from India for use in Tanganyika. A typical zebu with short horns, circular at base, convex profile, well developed dewlap and musculo-fatty thoracic hump.



16. *Shorthorned Zebu* (*Bengal*). Front and lateral views of 9th dorsal vertebrae of bull and cow sent by A. D. MacGregor, Esq., F.R.C.V.S.



17. *Longhorned Zebu* (*Amrut Mahal*) Bullock—Gunn, 1909—Note long horns and thoracic hump.

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*** Now out of print.**

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Anthelminthic Tests, Chiefly with Tetrachlorethylene, for the Removal of the Hookworm, *Gaigeria pachyscelis* from Infested Sheep, with Observations on the Effects of this Drug on Other Parasitic Nematodes.

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DURING the course of investigations by one of us (R. J. O.) on the life history, mode of spread and prevention of the sheep hookworm—*Gaigeria pachyscelis*—the writers have, when material became available, tried various drugs in an attempt to find a suitable medicant for the removal of this parasite from infested sheep. The drugs mainly experimented with were chemically pure Carbon Tetrachloride and Tetrachloroethylene, while n-Butyl-Chloride, Extract of Pyrethrum and other chemicals were also tried. The two first-named drugs have, since the researches of Hall and Shillinger, etc., been extensively used in the treatment of humans and dogs infected with hookworms, the former having been found by these workers and by Daubney (1930) to be also very effective in the treatment of sheep infested with the hookworm *Bunostomum trigonocephalum*. n-Butyl-chloride was tested by Wright and Schaffer (1932) and found to be 84 per cent. effective for the removal of hookworms from dogs. Pyrethrum extract was tried because of its known lethal action on cold blooded animals and its harmlessness on warm blooded animals, and in view of the favourable reports on its efficacy against hookworms in dogs by other authors.

In the first series of tests 18 adult Persian sheep were used, all of which had by faecal examination been found to carry a heavy infection of *Gaigeria pachyscelis*. These sheep had been bought from Mr. V. Theophilus, Pudimoe, where they had acquired a natural infection on his farm. Of these sheep ten were dosed with tetrachlorethylene in an equal volume of meal water, six were dosed with carbon tetrachloride in an equal volume of meal water and two were kept as controls. The following table gives the results:—

TABLE I.

Number of Sheep.	Drug.	Quantity.	Worms passed during next 3 days.	Eggs in faeces 12 days after drug.*	Remarks
36429....	C_2Cl_4	3 c.c.	—	††††	Died 13 days after dosing. 124 living Gaigeria collected.
36440....	,,	,,	—	††	—
36437....	,,	,,	9	††††	—
36442....	,,	4 c.c.	1	†††	Died 14 days after dosing. 123 living Gaigeria collected.
36426....	,,	,,	24	†††	—
36427....	,,	5 c.c.	—		—
36441 ...	,,	,,	—	†††	Died night after dosing. 77 live Gaigeria collected.
36428....	,,	,,	—	††††	—
36435....	,,	,,	—	†††	—
36441....	,,	10 c.c.	—	††††	
36438....	CCl_4	3 c.c.	—	††††	—
36433....	,,	,,	—	†††	—
36425....	,,	4 c.c.	—	†††	—
36436....	,,	,,	—	††††	Died 6 days after treatment. Worms not collected.
36431....	,,	5 c.c.	—	†††	
36434....	,,	,,	—	††††	—
36432....	Untreated.		—	††††	—
36443....	,,		—	†††	—

* † Few eggs present (1–10 on slide).
†† Fair number of eggs present (11–50 on slide).
††† Many eggs present (51–100 on slide).
†††† Numerous eggs present (over 100 eggs on slide).

Bags were tied on the sheep after dosing in order to collect all the faeces passed; the faeces were carefully washed and sieved and all the worms passed during the following three days were collected and counted. In order to determine the presence of eggs in the faeces, about 25 to 30 grams of fresh faeces were taken from each sheep either direct from the rectum or from the bag. These were then well broken up in tap water and then passed through several sieves to retain the coarser materials. Each was then placed in an Erlenmeyer flask and allowed to settle. The supernatent liquid was then carefully poured off and the remaining sediment was now mixed with an equal volume of glycerine and well shaken. It was then centrifuged in a tube 9 cm. deep and 3 cm. diameter, for about 3 minutes at about 1,000 revolutions pe rminute, when the eggs rose to the top of the liquid; these were then picked up with a flat bottomed glass rod about 1 cm. diameter and several (4 to 5) drops were transferred to a slide and examined microscopically. As the *Gaigeria* eggs are much larger and darker than those of the common round worms of sheep (*Haemonchus contortus*, *Trichostrongylus spp.* and *Oesophagostomum columbianum*) they could easily be recognised.

From Table I it will be seen that doses of 3 to 5 c.c. of Carbon tetrachloride had no effect on the removal of the worms; doses of 3 to 5 c.c. and 10 c.c. Tetrachlorethylene also had very little effect, although three of the sheep did pass out some worms, they nevertheless still retained a good infection; three sheep which died from one to fourteen days after dosing showed living hookworms on post-mortem, 124, 23 and 77 worms being collected respectively. The sheep which had received 10 c.c. passed no worms and still remained heavily infected. Sheep 36436 treated with carbon tetrachloride died six days after treatment and through a misunderstanding the worms were not collected. The two controls also passed no worms and remained heavily infested.

Previous to treatment the sheep had free access to hay and broken maize, and a week prior to treatment they daily received about 2 pounds of a 3 : 1 mixture of bone meal and salt, while maize was removed. Food or water was not kept away from them before or after treatment.

Three weeks after the above treatment six of the above sheep were again treated with Carbon tetrachloride and Tetrachlorethylene, and five with a mixture of equal parts of these drugs and two were kept as controls.

The followinog table gives the results:—

TABLE II

Number of Sheep.	Drug.	Quantity.	Worms passed during following 3 days.	Eggs in faeces 10 days after dosing.	Remarks.
36435...	C_2Cl_4	6 c.c.		†††	Killed following day. 39 living Gaigeria collected.
36444....	,,	,,		††††	
36438....	,,	,,	1	††††	
36437....	CCl_4	,,	--	†††	
36425...	,,	,,	1	†††	
36431....	,,	,,	—	†††	
36441...	CCl_4 and C_2Cl_4	,,	—	†††	
36428....	,,	,,	4	††††	Died 2 days later. 167 living Gaigeria collected.
36433....	,,	,,	--	†††	Died 2 days later. 65 living Gaigeria collected.
36434....	,,	8 c.c.	3	†††	
36426....	,,	,,	-	†††	
36432....	Untreated control.		—	††††	
36443....	,,	,,	-	†††	

Except for the last two sheep treated, the dose was in all cases mixed with 12 c.c. meal water; in the last two cases only 10 c.c. meal water was added. Since the first treatemnt all the sheep had free access to hay and the salt and bone-meal mixture, also they were not kept away from food or water before and after dosing. They were all in a very poor condition.

Table II shows that 6 c.c. of Carbon tetrachloride or Tetrachlorethylene in meal water had very little or no effect on the removal of the hookworms, two worms only being removed and all the sheep still remaining heavily infested as shown by egg flotation. One of the sheep which was very weak was killed the following day, and on post-mortem 39 living *Gaigeria* were found adhering to the mucous membrane. Of the 5 sheep which received 6 and 8 c.c. of a mixture of equal amounts of these two drugs 2 sheep passed 4 and 3 hookworms respectively during the following 3 days. Two of them which died two days after treatment still had 167 and 65 living *Gaigeria* respectively, some of which were lying free in the lumen of the intestine. The two controls passed no worms and still remained heavily infected. It would thus appear that a mixture of these drugs given in 6 and 8 c.c. doses is not effective for the removal of *Gaigeria* hookworms.

As the sheep were in a very poor condition they were from now on fed on hay and a liberal supply of crushed maize plus bone-meal and salt. They picked up gradually in condition, and during the course of the next seven weeks a number gradually lost most of their infection and two eventually became free of hookworms. Centrifuge egg flotation determinations after 6 and 7 weeks on this diet and faecal culture after 6 weeks gave the results shown in the following table:—

TABLE III.

Number of Sheep.	Gaigeria eggs after 6 weeks.	Gaigeria eggs after 7 weeks.	Culture.	G.	O.c.	H.c.	Tr.	S.p.*
36425.......	—	—	—	—	—	—	—	—
36426.......	†	†	W	20	34	—	14	32
36431.......	†	†	—	—	—	—	—	—
36432.......	†††	††	M	79	5	—	12	4
36434.......	†	—	M	16	22	—	55	7
36437.......	†††	†††	M	12	—	—	35	53
36438.......	†††	†††	M	62	10	—	22	6
36441.......	††	††	W	30	14	—	56	—
36443.......	††	††	M	50	10	—	30	10
36444.......	††	††	M	47	1	—	52	-

* W=weak culture; M=medium culture. These refer to the density of the larvae on the sides of the bottle 8–10 days after culture.

G=Gaigeria; O.c. Oesophagostomum; H.c.=Haemonchus; Tr.=Trichostrongylus; S.p.=Strongyloides. The numbers give the percentage of the different kinds in the first 100 larvae counted.

For some inexplicable reason no larvae developed in the culture of faeces from sheep 36431, although eggs were present.

When comparing these intensities of hookworm infection, as revealed by centrifuge egg flotation determination, with those of the same sheep seven weeks previously we find that two sheep have entirely lost their infection (††† to –), two have lost two-thirds of their infection (††† to †), two have lost half their infection (†††† to ††), two have lost one-third of their infection (††† to †) one has lost a quarter of its infection (†††† to †††) and one has retained its whole infection.

These sheep had now been in this experiment for four months and during this period they had been kept in a dry pen and were consequently not exposed to reinfection. The infection they still retained was that which they had brought with them. The diminution of this infection may thus be ascribed to either of two causes, firstly that many of the parasites had already reached the end of their span of life and thus naturally died, only the younger hookworms remaining; or secondly by better feed the constitution of the sheep was improved with the result that they were able to rid themselves naturally from a considerable portion of their infection. It is a fact often seen in the field that where the diet of sheep is improved, the sheep, with the improvement of their condition, tend to rid themselves of a large proportion of their worm burden. In the above case both the above-mentioned causes may have been responsible, but the writers are inclined to the view that the better diet played the principal role.

As the sheep appeared to be rapidly losing their infection and as the treatments with Carbon tetrachloride and Tetrachlorethylene had given such disappointing results it was decided to dose six of the still infected sheep with 1 to 5 c.c. of Stafford Allen's extract of Pyrethrum M.225, one sheep with 15 c.c. C_2Cl_4 and to keep one sheep as a control. In all cases the dose was mixed with 20 c.c. of distilled water and was administered by stomach tube. The results are set out in the following table:—

TABLE IV.

Number of Sheep.	Drug.	Amount.	Number of worms passed during 3 days following.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.
36443............	Ext. Pyr.	1 c.c.	—	††	††
36437............	,,	,,	—	††	††
36440............	,,	3 c.c.	—	†	—
36441............	,,	,,	—	††	†
36431............	,,	5 c.c.	—	†	†
36438............	,,	,,	—	†††	†††
36444............	C_2Cl_4	15 c.c.	3	††	††
36432............	Untreated Control.		-	††	††

From this table it is evident that the intensity of worm infestation remained about the same before and after treatment with Extract of Pyrethrum; it is true that after 10 days one of the sheep (36440) which had received 3 c.c. had lost its infection († to -) and the other (36441) which had received the same dose had its infection halved (†† to †); no worms, however, were passed during the ensuing three days after treatment so that the loss probably took place subsequently and before the tenth day after treatment; this loss was probably due to natural causes. It thus appears legitimate to conclude that a dose of 1 to 5 c.c. of Extract of Pyrethrum in water, dosed direct into the rumen has no effect on the expulsion of the *Gaigeria* hookworm.

The sheep which had received 15 c.c. of C_2Cl_4 in water direct into the rumen passed 3 hookworms, but the infestation remained the same. The control sheep showed no change.

As the hookworm infestation of these sheep had now become rather low it was decided to reinfect them artificially. Unfortunately during this interval several of these sheep died. The remaining few were not further experimented with except one which was very weak. This sheep was dosed with 10 c.c. C_2Cl_4 in 10 c.c. liquid paraffin and died two days later. No worms were passed and at post-mortem 334 living *Gaigeria* were found still adhering to the small intestine and eleven were free in the large intestine, of which seven were still alive. The other sheep were set aside in order that cultures may be made from them to infect fresh sheep.

While a stock of infested sheep was being prepared, the method of administering drugs into the abomasum of sheep, after a preliminary dose of copper sulphate solution, became known (Ross, 1934; Mönnig and Quin, 1935), and it was decided to try this method in future experiments, again using Tetrachlorethylene and also n-Butyl-Chloride. Nine sheep were eventually obtained which showed a moderate infestation by the egg flotation method. They were divided into three groups of three each; one group received 5 c.c. C_2Cl_4 in 10 c.c. liquid paraffin, the other 10 c.c. C_2Cl_4 in 20 c.c. liquid paraffin and the third group 10 c.c. n-Butyl-Chloride in 20 c.c. liquid paraffin. Immediately prior to dosing each sheep was drenched with about 10 c.c. of a 2 per cent. copper sulphate solution. The sheep were not specially prepared, neither was food or water kept away from them before or after dosing. The results of this treatment are shown in the following table:—

TABLE V.

Number of Sheep.	Drug.	Amount.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.	Worms passed during following 3 days.
39173............	C_2Cl_4	5 c.c.	†††	†††	—
39588............	,,	,,	††	††	—
39998............	,,	,,	††	††	—
39724............	,,	10 c.c.	††	—	28
39996............	,,	,,	††	—	19
40009............	,,	,,	††	—	23
39184............	n. But. Chl.	,,	††	†	5
39477............	,,	,,	††	—	17
39731............	,,	,,	††	††	—

Twenty days after treatment the faeces from the three sheep which had been treated with 10 c.c. Tetrachlorethylene were again axamined and were again found negative for hookworm eggs.

The results of this dosing appear to show that when dosed immediately after drenching with copper sulphate solution a dose of 5 c.c. Tetrachlorethylene is not effective for the removal of *Gaigeria* hookworms; a dose of 10 c.c. n-Butyl-Chloride is about 50 per cent. effective and a dose of 10 c.c. Tetrachlorethylene is very effective. In order further to test the effectiveness of this drug the four sheep which had passed no worms in the above test were each dosed with 10 c.c. Tetrachlorethylene in 20 c.c. liquid paraffin immediately after drenching with a weak copper sulphate solution with the following results.

TABLE VI.

Number of Sheep.	Drug.	Amount.	Eggs in faeces at treatment.	Eggs in faeces 10 days after treatment.	Eggs in faeces 20 days after treatment.
39173.......... ...	C_2Cl_4	10 c.c.	†††	—	—
39588............	,,	,,	††	††	††
39998............	,,	,,	††	—	—
39731............	,,	,,	††	††	††

The worms passed were unfortunately not collected in these cases.

This result, although not so promising as the previous one, shows that in two sheep all the hookworms were removed while in the remaining two the hookworm burden was not reduced. In these two tests we thus have five sheep out of seven freed of their hookworms. What is especially striking is that these worms were either entirely cleared or not at all. This leads one to suspect that in the five cleared sheep the drug passed direct into the abomasum and was thus able very soon to reach the hookworms in the intestine, whereas the drug passed into the rumen in the other two sheep, and consequently was not able to come in contact with the hookworms immediately and in concentrated form. These sheep also carried a fairly heavy burden of wireworms and nodular worms and it was striking that in the five cleaned sheep the number of strongyle eggs was also reduced.

Having obtained these highly promising results with the dosing of Tetrachlorethylene immediately after drencing with a 2 per cent. solution of Copper sulphate, an opportunity was awaited to try out this method of treatment on a large scale. This opportunity soon occurred in that Mr. Theophilus, who had been suffering very extensive losses in his Persian sheep due to this hookworm, offered to place his entire flock of about 1,000 sheep at our disposal if we wished to carry out any tests. This offer was accepted and one of us (R. J. O.) proceeded to his farm in the Vryburg district of the Cape Province. His adult sheep, about 480 in number, were found to be in a very bad state, very thin and very anaemic and a large percentage of them showed marked swellings under the jaw. Six full grown ewes of these were taken and an examination of their faeces, taken from the rectum, showed that they were very heavily infested with the Gaigeria hookworm and also with strongyloids. They were then dosed with 10 c.c. Tetrachlorethylene mixed in an equal volume of a light neutral mineral oil after drenching with 10 c.c. of a 2 per cent. copper sulphate solution. Two days after they were slaughtered with the following results:—

TABLE VII.

No. of Sheep.	Drug.	Amount.	Hookw.	H.c.	Tr	O.c.	Remarks.
1....	C_2Cl_4	10 c.c.	-	—	—	Numerous	All living.
2....	,,	,,	Many	Numerous	Numerous	,,	,,
3....	,,	,,	—	—	—	,,	,,
4....	,,	,,	Numerous	Many	Fair No.	,,	,,
5....	,,	,,	1 (detached in s. int.)	—	—	,,	,,
6....	,,	,,	Numerous	Many	Many	Many	,,

The above test showed that drenching as above cleaned half of the sheep of their hookworms, whereas the remaining three still remained heavily infested. In the three cleaned sheep wireworm and trichostrongyles were also absent, whereas in the remaining three both these worms were present. All six sheep were still heavily infested with nodular worms. It would thus appear from this test that where the drug is able to pass direct into the abomasum it kills not only hookworms but also wireworms and trichostrongyles. In the three sheep which remained infested the drug most probably passed direct into the rumen.

The sheep were dosed in the cool of the evening directly from the veld. After dosing, which was done by means of a suitable syringe with a long bent nozzle and squirting the drug gently down the side of the cheek, all the sheep showed marked reactions; all became somewhat giddy and sluggish and three of them developed marked tympany of the rumen which had eventually to be relieved by puncturing with a trocar. Next morning, however, all six were normal again and grazing.

Sixty sheep were now taken and divided into two groups of thirty-five and twenty-five sheep; their ages varied from 2-tooth to adults, and consisted of ewes only. Faeces from twenty-five were taken from the rectum and all on examination showed *Gaigeria* eggs. As these sheep had not been selected from the flock but taken at random, except that all lambs were discarded, it appeared legitimate to conclude that if not all then a very high percentage of the adult sheep carried hookworm infection. In addition all the sheep showed numerous strongyle eggs, probably *H. contortus*, *Trichostrongylus sp.* and *Oesophagostomum columbianum* as revealed by the post-mortems on the sheep discussed above. The group containing thirty-five sheep was given each 5 c.c. C_2Cl_4 in an equal volume of a light mineral oil, while the other group received 10 c.c. C_2Cl_4 in an equal volume of light mineral oil. Immediately prior to dosing with this drug each sheep received 10 c.c. of a 2 per cent. copper sulphate solution. These sheep were dosed during the late afternoon direct from the veld; during the evening it was noticed that some of the sheep in the group which had received the larger dose were slightly bloated and a trocar had to be used on one; the sheep in the other group showed no signs of bloating, although, after dosing, both groups became somewhat giddy and sluggish. Next morning, however, all the sheep were again normal. Two of the sheep from each group were now slaughtered, 13 hours after dosing, with the following results.

TABLE VIII.

Drug.	Amount.	Worms at post mortem.			
		Gaigeria.	H.c.	Tr.	O.C.
C_2Cl_4	5 c.c.	Many	Numerous	Numerous	Numerous
,,	,,	,,	,,	,,	,,
,,	10 c.c.	-	—	—	,,
,,	,,	—	—	-	,,

The two sheep which had received the larger dose showed no hookworms in the small intestine. Among the faeces of the large intestine, however, numerous dead hookworms were found. The other two sheep showed no dead hookworms in their faeces.

The above experiment confirmed the results obtained at the laboratory (vide Table V), namely that a dose of 5 c.c. C_2Cl_4 was not sufficient to remove the hookworms, whereas that of 10 c.c. was very effective. A large number of sheep was used in this experiment in order to see whether bloating was generally associated with the administration of the drug, and if so whether it had to be considered a serious reason against its use. From the above result the writers are inclined to the view that sheep after adequate dosing should be carefully watched during the ensuing few hours and any sheep showing marked bloating should be relieved; notwithstanding this drawback, it is felt that, with careful handling, Tetrachloretylene is a relatively safe drug for use, especially for the reason that since performing the above test, a large number of doses have been administered at the laboratory to sheep of different ages, in poor and good condition, starved and unstarved, watered and unwatered, with and without copper sulphate solution, but in not a single case was there any signs of bloating. (See also discussion on toxicity of Tetraethylene below.)

For the next test thirty-eight sheep similar to and from the same flock as those used in the foregoing test were used. These had slept overnight in a kraal adjacent to the sheep used in the foregoing test, and were dosed the next morning after drenching with Copper sulphate solution. Each sheep received 20 c.c. of a half-and-half mixture of C_2Cl_4 and light mineral oil; these sheep were then given free access to water, which they did not take, and placed in a nearby paddock with plenty of green grass. For a few hours these sheep kept together and appeared very drowsy, but otherwise no serious after-effects were noted and by midday all were normal and grazing.

This experiment thus tended to show that sheep which had been kraaled overnight and dosed next morning, were less likely to bloat than those which had been dosed during the late afternoon direct from the veld.

The results so far obtained in the field with Mr. Theophilus' sheep may be summarised as follows:—

1. Six selected adult sheep and twenty-five adults taken haphazardly showed by faecal examination that they were badly infested with hookworms; the probability was thus that if not the whole adult flock then a very high persentage of them was infested.

2. All these sheep were also heavily infested with wire-, nodular- and trichostrongyle worms.

3. Of eight sheep which had received 10 c.c. C_2Cl_4 five had on post-mortem lost all their hookworms, wireworms and trichostrongyle worms. The remaining three still had all these worms. All eight still had numerous nodular worms.

4. Of two sheep which had received 5 c.c. C_2Cl_4 both on post-mortem still carried a heavy infection of all the abovenamed worms.

5. It appears safer to dose the sheep in the morning after having been kraaled overnight.

6. As the sheep which had become cured of hookworms had lost their entire worm burdens of the abomasum and small intestine, and as the non-cured appeared to have retained all these, it appears ligitimate to conclude that the effectiveness of Tetrachloiethylene is almost entirely dependent on whether it reaches the abomasum direct or not. This conclusion is substantiated by the results obtained by the dosing of this drug without previous drenching with a Copper sulphate solution, where the effectiveness of this drug was shown to be very low.

As it had now been shown that Tetrachlorethylene was a very effective anthelmintic for the treatment of *Gaigeria* hookworm, provided it is dosed direct into the abomasum, it was decided to dose the whole flock, in batches of about two hundred every morning. Eact adult sheep was to receive the full dose, i.e. 10 c.c. $C_2Cl_4 + 10$ c.c. neutral mineral oil. The dosing had to be repeated three times at intervals of 10 to 14 days, and 10 days after each dosing 50 or more faeces samples had to be taken from the rectum from a similar number of sheep taken from the flock at random; these faeces to be examined miscrscopically by the egg centrifuge flotation method for the presence or absence of hookworm eggs. It was considered that such a procedure would give a good indication of the progress of the cure or otherwise in the whole flock after one, two or three treatments. As twenty-five sheep taken at random had already shown to be all infected with hookworms and other strongyloid worms, it appeared legitimate to assume that prior to treatment all, or a very high percentage of the sheep, were infected with both hookworms and other strongyloids.

The dosing of the first ninety sheep on the first day was done personally and it was arranged that the owner should continue the first dosing and also carry out the required second and third treatments. The collection of the faeces was also done under the supervision of the owner, when 25 to 30 grams from each sheep were placed in a suitable tube in 10 per cent. formalin and sent to the laboratory for further examination. As the sheep from which the samples were taken were on each occasion taken at random it is possible that samples from the same sheep may have been collected on more than one occasion; the chances, however, are that on each occasion the sheep were different, or at least the greater number of them were.

The results after these treatments may be summarised as follows:

After 1st treatment of 54 samples taken*:—

23 (42·6 per cent.) were free of hookworm eggs (–).
15 (27·8 per cent.) were just positive for hookworm eggs (†).
3 (5·5 per cent.) had a light infection of hookworms (††).
9 (16 6 per cent.) had a fair inefction of hookworms (†††).
4 (7·4 per cent.) had a good infection of hookworms (††††).

In addition all the sheep showed the presence of a considerable number of other nematode eggs (††† to ††††).

After 2nd treatment of 50 samples taken from the whole flock:—

38 (76 per cent.) were free of hookworm eggs (–).
3 (6 per cent.) were just positive for hookworm eggs (†).
5 (10 per cent.) had a light infection of hookworms (††).
2 (4 per cent.) had a fair infection of hookworms (†††).
2 (4 per cent.) had a good infection of hookworms (††††).

With regard to other helminths these samples showed that:—

16 (32 per cent.) were free of all helminth eggs (-).
5 (10 perc ent.) were just positive for helminth eggs (†).
16 (32 per cent.) showed a light helminth infection (††).
6 (12 per cent.) showed a fair helminth infection (†††).
7 (14 per cent.) showed a good helminth infection (††††).

After the 3rd treatment of 54 samples taken from the whole flock:—

54 (100 per cent.) were free of hookworm eggs (–).
45 (83·3 per cent.) were free of all helminth eggs (–).
4 (7·4 per cent.) were just positive for helminth eggs (†).
3 (5·5 per cent.) showed a light helminth infection (††).
2 (3·7 per cent.) still had a fair helminth infection (†††).

(*These samples were taken at random from the 90 sheep dosed personally.)

For practical purposes we can consider that the sheep which are just positive for hookworms (i.e. from 25 to 30 grams of whose faeces after sedimentation and centrifuging not more than 10 eggs are seen in 4 to 5 drops from the top of the centrifuge liquid) are also ucred, as the few worms which they contain would not produce any clinical symptoms. We have then a percentage of sheep cured of hookworms of 70·4 per cent. after the first treatment, 82 after the second treatment and 100 after the third treatment; in addition we find a percentage cure from other helminths of 42 after the second treatment and 90·7 after the third treatment. If these figures, which statistically are highly significant, are indicative of the flock, then the efficacy of Tetrachlorethylene, dosed immediately after the administration of a 2 per cent. solution of Copper sulphate, is very satisfactory. (Fig.) In addition the margin of safety is very high, as some 1,400 doses were administered with only 7 deaths, all of which were acidental and due to the direct result of the passage of either the Copper sulphate solution or Tetrachlorethylene direct into the lungs. It is true that bloating was often a sequel of dosing, but when the sheep are dosed in the morning, after having been kraaled overnight, this reaction is not serious; besides it is doubtful whether this bloating can be considered to be a general sequel of Tetrachlorethylene administration; it is possible that some ingredient in the feed in this particular area is a predisposing cause, perhaps vermeerbossie (*Geigeria* spp.) which is very plentiful in this area.

In order that these highly significant results could be confirmed or not the treated sheep were personally examined 25 days after the last dosing and 50 faeces samples were again taken from different sheep. The improvement in the general condition of the treated ewes was striking; whereas prior to treatment they were all very thin, weak and anaemic, they were now in a very good and strong condition. Only three sheep in the whole flock were still poor, and one of these, the only one in the flock, showed clinical symptoms of verminosis (anaemia and swollen jaw). A post-mortem of this sheep showed about 50 hookworms and a good number of wireworms and nodular worms. Faeces from the other two showed no hookworm eggs on examination, but both still had a light infection with other worms. The 50 samples, taken at random, included faeces from these three sheep, and on examination the following results were obtained:—

47 (94 per cent.) free of hookworms (–).

1 (2 per cent.) just positive for hookworms (†).

2 (4 per cent.) fair infection with hookworms (†††).

27 (54 per cent.) free of all other worms (–).

15 (30 per cent.) had a very light infection with other worms (†).

4 (8 per cent.) had a light infection (††).

2 (4 per cent.) had a fair infection with other worms (†††).

2 (4 per cent.) had a good infection with other worms (††††).

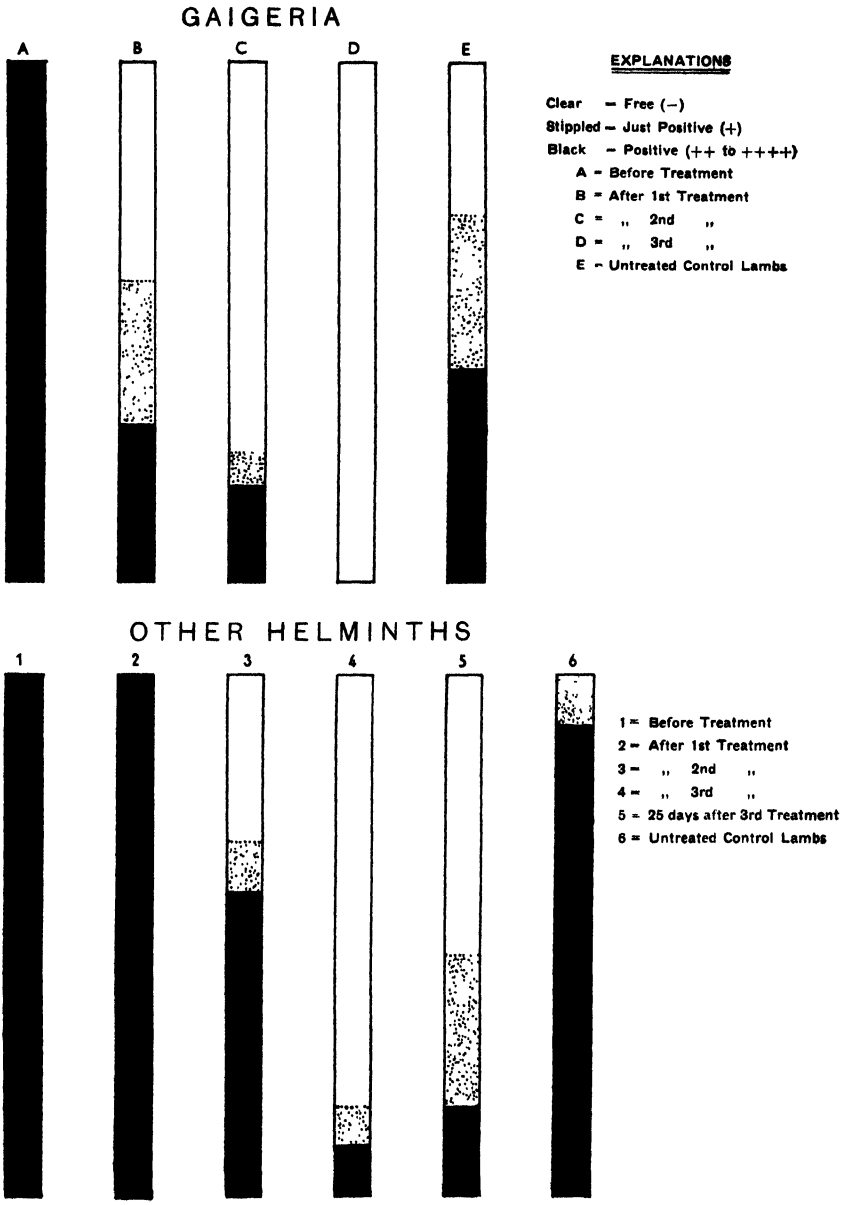

Fig. 1. Columns showing worm burden (*Gaigeria* and other Helminths) of flock before and after treatments with Tetrachlorethylene.

From these results it will be seen that whereas in the previous faeces samples taken 10 days after the third dosing all were free of hookworms in these latter, taken 25 days after the last dosing three showed hookworms eggs This is not due to reinfection as it takes about 10 weeks for these hookworms to attain the egg laying stage in the host; the only explanation is that these sheep were infected prior to treatment and that as far as they were concerned the treatment was not successful, in that the drug was, at least in two of them, not swallowed direct into the abomasum. That the previous samples were all negative was simply a matter of chance, as the sheep from which they were taken were not selected. Taking the combined results of these two faecal examinations we still obtain very significant results, namely out of 104 samples there are 101 free of hookworms, and only 3 still infected, a percentage absolute cure of just over 97, which is very satisfactory.

That the loss of infection was not due to a natural loss of the hookworms is shown by about one hundred lambs which were running with the ewes; faecal examinations of ten of them showed that seven of them harboured a light hookworm infection at the time the last faeces samples were taken from the treated ewes. It may thus justly be concluded that these latter samples fully confirm the results of those taken previously; this view is also supported by post-mortem examinations done by the owner on four sheep which had died as a result of eating vermeerbossie (*Geigeria* sp.) a few days after the taking of the third batch of faeces samples; in these sheep there were no hookworms; neither were there any wireworms or trichostrongyles; three of the sheep were also free of nodular worms and one had very few.

As far as the other worms are concerned, the results obtained from an examination of this last lot of faeces is also very striking and supports the previous results. It is true that the infestation is now higher (54 per cent. free as against 83 per cent. free) but the intensity is not very much greater and can be very easily explained as due to reinfection; the period of 25 days since the last dosing is quite sufficient for wireworms and trichostrongyles to infect sheep and attain the egg-laying stage. The intensity of infestation with these worms is, however, very much lower than that in the one hundred lambs running with the ewes or in a flock of about three hundred and fifty lambs weaned some four months previously and running separately. As this infection was a mixed one, consisting of wireworms, trichostrongyles and nodular worms, and as the ewes harboured all these worms to a very marked extent prior to dosing, we are forced to the conclusion that their dimunition is due to the dosing with Tetrachlorethylene.

That this drug has a toxic effect on hookworms, wireworms and trichostrongyloids is very easily appreciated, because, when dosed direct into the abomasum, the drug immediately or very soon comes into contact with these helminths in a very concentrated condition and its action is then very quick; in two sheep post-mortemed thirteen hours after dosing, no worms were found in the abomasum or small intestine, but numerous hookworms were present in the large intestine up to the anus; in ninety sheep, whose droppings were examined four hours after dosing, a number were already passing dead hookworms. In both cases no search was made for wireworms or trichostrongyles. Its role, however, in the expulsion of the nodular worms is not so clear, and its effects on these worms became apparent only after the second and third dosings; it is possible that after the initial removal of the hookworms, wireworms and trichostrongyles, the balance between the nodular worms and their host became disturbed with the result that the worms were naturally go rid of. During the whole course of treatment no scouring of the sheep was noticed which would naturally tend to rid the sheep of a portion of its nodular worms.

While these experiments were being carried out, more detailed information became available with regard to stimulation of the oesophageal reflex and, in view of the fact that it appeared necessary to use 10 per cent. $CuSO_4$ for satisfactory stimulation in full-grown, poor-conditioned sheep, tests with 5 c.c. and 7·5 c.c. Tetrachlorethylene were again started on this basis. The following sheep were dosed with a mixture of equal quantities of the drugs and liquid paraffin, after a preliminary dose of 2·5 c.c. 10 per cent. $CuSO_4$.

Dose of C_2Cl_4.	Eggs at treatment.	Eggs 14 days after treatment.
5 c.c.	†	†
,,	†	—
,,	†	—
,,	†	—
,,	†††	—
,,	††	††
,,	††	†
,,	††	—
,,	††	—
7·5 c.c.	†††	††
,,	††	†††
,,	††	—
,,	††	—

From these results it is seen that six out of nine sheep were freed of *Gaigeria* infestation by a single treatment with 5 c.c. of the drug, while two out of four were cleaned with 7·5 c.c. It therefore appears to be desirable to continue investigations in this direction in order to reduce the costs and risks of this treatment.

THE TOXICITY OF TETRACHLORETHYLENE.

Information on the toxicity of this drug for sheep is urgently required. The experiments reported above and the experience of farmers have shown that the drug has two properties which are obviously undesirable. In the first place the drug is volatile and, even when given in mineral oil as a vehicle, sheep are very liable to cough and choke when the administration is not carried out with great care. Secondly, absorption from the small intestine takes place very rapidly, since the sheep show signs of giddiness within a few minutes of treatment, even if they have swallowed the mixture satisfactortly, and the drug can be smelt in their breath.

The first difficulty cannot be obviated by administering the drug in a capsule, since it would then not be swallowed into the abomasum. Consequently attempts were made to find a better vehicle. Such a vehicle should comply with the following requisites: (1) It should bind the drug in such a way that no fumes escape at the moment of administration; (2) it should be inactive itself and should not be absorbed from the abomasum or intestine; (3) it should reduce absorption of the drug without binding the latter in such a way that it becomes less effective against the parasites. (It is known that some vehicles may enter into such a close combination, e.g. solution, with anthelminthics that the efficacy of the latter is reduced.) This third requisite is the most difficult to satisfy, but it would be worth while to investigate in this direction, since Tetrachlorethylene may become a satisfactory anthelminthic for eosophagostomes also if success could be achieved.

At first a series of heavy mineral oils, right up to vaseline, were tried and the heavy oils did to some extent satisfy points 1 and 2, but not 3, although efficacy was not reduced.

It was then found that Tetrachlorethylene (also Carbon tetrachloride) could easily be emulsified in the following way: To 25 c.c. of an aqueous solution of 7·7 gm. soft soap add successive small quantities of a mixture containing 37·5 c.c. Tetrachlorethylene and 37·5 c.c. liquid paraffin, while shaking vigorously. The emulsion obtained resembles thick cream and can be diluted with half a volume of soft water. It will be seen that 40 c.c. of the diluted emulsion contains 10 c.c. Tetrachlorethylene. This emulsion can be administered without the slightest risk of coughing or choking on account of fumes, because these are not given off. This satisfies point 1 completely. The emulsion is, however, broken by the acid in the abomasum and does therefore not have any further advantage. The sheep treated with 5 c.c. and 7·5 c.c. Tetrachlorethylene, reported on in the last test mentioned above, were dosed with this emulsion. Also in other cases it was found that the efficacy of the emulsion is quite satisfactory.

Continuing investigations in this direction it was found that emulsions could be made with gum arabic using various proportions of the drug and mineral oil, e.g. gum arabic 9 gm., water to 50 c.c. C_2Cl_4 50 c.c., liquid paraffin 25 c.c. Such an emulsion is not broken by acid and apparently passes through the abomasum. On reaching the small intestine it is, however, even more rapidly absorbed than a solution of the drug in mineral oil, causing more severe symptoms of giddiness. It has, however, the advantage that it can be mixed with solutions of magnesium sulphate or tannic acid, which would tend to decrease absorption . And indeed it was found that such combinations appeared to satisfy the requirements enumerated above, except that the efficacy against *Gaigeria* appeared to be greatly reduced.

It was further found that the addition of a small quantity of alkali (NaOH or $NaHCO_3$) to the gum arabic emulsion led to the rapid development of tympanitis in a fair proportion of the sheep, some dying as a result.

Returning to the soap emulsion it was found possible to add croton oil to the undiluted emulsion and then diluting as before. Sheep dosed with this preparation showed very little giddiness, the majority remaining quite normal after treatment. Doses of 1 and 2 c.c. croton oil were used. A treatment carried out on sheep infested with *Gaigeria*, using an emulsion as described above and containing in each dose 10 c.c. C_2Cl_4 and 1 c.c. croton oil gave the following result:—

Sheep.	Gaigeria and faeces passed in two days after treatment.	Eggs at treatment.	Eggs 14 days later.
1	3 S×N*	††	—
2	28 S×N	††††	—
3	21 S×N	†††	+
4	— S×N	††	—
5	20 N	††††	—
6	16 N	††	—

* S=soft. N=normal.

In tests on sheep infested with *Oesophagostomum columbianum* it seemed as if the efficacy was greater than that of an ordinary solution of Tetrachlorethylene and mineral oil.

The above are only preliminary records of the work done in connection with the undesirable effects of Tetrachlorethylene and the investigations will be continued.

SUMMARY.

1. Doses of 3, 4, 5, 6, 10 and 15 c.c. of pure Tetrachlorethylene per sheep are not efficient for the removal of the hookworm—*G. pachyscelis*.

2. Doses of 3, 4, 5 and 6 c.c. pure Carbon tetrachloride are not efficient for the removal of the hookworm—*G. pachyscelis*.

3. A dose of 8 c.c. of a half-and-half mixture of Tetrachlorethylene and Carbon tetrachloride is not efficient for the removal of the hookworm—*G. pachyscelis*.

4. Doses of 1, 3 and 5 c.c. of Stafford Allen's Extract of Pyrethrum M.225 had no effect in the removal of hookworm—*G. pachycelis*.

5. When dosed into the abomasum after administering 10 c.c. of a 2 per cent. Copper sulphate solution, 5 c.c. of Tetrachlorethylene in mineral oil was ineffective whilst 10 c.c. was very effective for removal of the hookworm—*G. pachycelis.*

After 2·5 c.c. of 10 per cent. Copper sulphate a dose of 5 c.c. was fairly effective.

6. n-Butyl chlordie in 10 c.c. doses was only partially effective for the removal of the hookworm—*G pachyscelis*—when dosed after Copper sulphate solution.

7. In a flock of sheep badly infested with this hookworm and also with wireworms, trichostrongyles and nodular worms 10 c.c. pure Tetrachlorethylene in an equal volume of liquid paraffin administered three times immediately after drenching with 10 c.c. of 2 per cent. $CuSO_4$ solution at intervals of 10 to 14 days was very effective for the removal, directly or indirectly, of all these worms, giving a percentage cure of 97 for hookworms and 83 for all the other worms.

8. The undesirable effects of Tetrachlorethylene during administration, i.e. coughing and choking, can be overcome by using a soap emulsion of the drug.

9. Experiments carried out with a view to overcoming the rapid absorption of the drug from the alimentary tract and its sequelae, i.e. giddiness and reduced efficacy, are described.

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A New Fly Repellent and a Blowfly Dressing.

Preliminary Report.

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EVERYONE who is acquainted with the problem of myiasis in sheep and other animals and also in man, realises the importance of a satisfactory fly repellent, and much work has been done, particularly by Bishopp and his associates in the United States, to find a suitable substance.

In a search for fly repellents the author has examined some oils of common South African plants and wishes to record in this article some preliminary work on one such oil, which appears to be a very effective blowfly repellent, and also to report on tests of certain chemicals which are very toxic to maggots and which could be used in combination with a repellent for the treatment of myiasis.

TAGETES OIL AS A FLY REPELLENT.

The plant *Tagetes minima* (popularly known in South Africa as "khaki bush") is a common weed which is a cause of trouble in maize and other lands in most parts of the Union. It grows on poor, dry soil to a height of a few inches while, in more favourable circumstances, it may reach a height of seven feet and the main stem bears a number of branches, each developing a cluster of small, pale yellow flowers at its tip. If the plant is cut it develops new branches and usually forms a more dense growth than before. The flowering season is towards the end of summer and during the winter the plants die off.

This plant contains a strongly-smelling volatile oil, or rather a mixture of oils, in its leaves, flowers and seeds. The oil can easily be obtained by steam distillation and the yield of the flowering plant is about 0·5 per cent. of the total weight.

A few years ago it was maintained by a certain writer in the daily press that this plant contains an active principle which was effective for treatment against internal and external parasites. The present author made careful tests with the distilled oil and also with infusions and decoctions of the whole plant against internal parasites such as *Haemonchus contortus* and other gastro-intestinal parasites of sheep and, together with Mr. Bedford of this Institute, tested the material against *Psoroptes communis ovis* and *Melophagus ovinus* on sheep and ticks on calves. In all cases the results were entirely negative with the exception of a slight anthelminthic effect in some cases.

In order to test the fly repellent properties of the oil various experiments were made.

A. TESTS WITH TREATED BAIT.

1. The first test was made by dividing the liver of a slightly decomposed sheep carcass into two equal parts and placing each half into a large, shallow glass dish. Specimen A was the untreated control; 10 drops of Tagetes oil were dropped on different parts of B and the oil soon spread over the whole surface of the liver. The two dishes were placed in the broken shade of trees where blowflies were plentiful. Two days later specimen A was attracting large numbers of flies and contained numerous eggs and young maggots, while B was still quite free of flies, eggs and maggots and the smell of the oil was still quite strong.

2. The second test was made in a way similar to the method used by Bishopp and his co-workers. Glass beakers of one litre capacity were used as containers. Into each was placed moist sand to a height of 5 cm., and on this 60 gm. liver and 60 gm. intestines of a slightly decomposed sheep carcass. The bait was sprinkled over with 1 c.c. of the repellent tested, attempting to spread the fluid as evenly as possible over the bait. The beakers were placed 6 feet apart under a thatch roof open on all sides. Two beakers were prepared for each repellent and there were three controls; they were so arranged as to get the best possible balance, taking into account the effect of light and the possible influence of one specimen on the other. The results are given in the following table:—

		Tagetes Oil.			Oleum Picis (Commercial)			Controls.		
		Flies.	Eggs.	Maggots.	Flies.	Eggs.	Maggots.	Flies.	Eggs.	Maggots
22/1/35	12.0 p.m.	Specimens were set out.								
22/1/35	4.0 p.m.	0	0	0	2M.d./2	0	0	5M.d./3	0	0
23/1/35	9.30 a.m.	0	0	0	1M.d./1	0	0	0	0	0
		Weather cloudy, cool wind.								
	12.0 p.m.	0	0	0	2M.d./1	0	0	2L.s./ 8M.d./3	0	0
		Clouds broken, temperature rising.								
	2.0 p.m.	0	0	0	2L.s./2 2M.d./	+/2	0	6L.s./3 10M.d./3 1S.h.	+3	S.h./1
24/1/35	9.30 a.m.	0	0	0	1L.s./1 4M.d./2	+/2	+/1	9M.d./3	+/3	+/3
	12.0 p.m.	1L.s./1 1M.d./	+/1	0	1L.s./1 5M.d./s	+/2	+/1	3L.s./3 11M.d./	+/3	+/3
	2.0 p.m.	2M.d./1	+/1	0	4M.d./2	+/2	+/2	3L.s./ 21M.d./	+/3	+/3
25/1/35	9.30 a.m.	0	+/1		15L.s./ 20M.d./2	+/2	+/2	20L.s./ 25M.d./3	+/3	+/3

M.d. = *Musca domestica*.
L.s. = *Lucilia sericata*.
S.h. = *Sarcophaga haemorrhoidalis*.
2M.d./2 = 2 M.d. at 2 beakers.
+/1 = Eggs or larvae present in one beaker.

It will be seen that the tagetes oil was very effective, particularly in comparison with oleum picis. The latter does not show up well because the quantities of the repellents used were small, but this was done since it had been determined in other similar tests made previously (not here recorded) that the tagetes oil appeared to be stronger than oleum picis in regard to repellent properties for blowflies.

It is not the intention to record in this preliminary report all the tests that have been made, but to give a few typical results obtained after the first tests made for orientation had been carried out. The work is being continued in various directions and more detail could be given in a later article.

B. TESTS IN OLFACTOMETER.

Since Tagetes oil has a very strong odour it was thought that this might simply mask the odour of baits used and it was therefore decided to test whether the oil has definite repellent properties for flies.

The type of olfactometer described by Krygsman (1930, 1931) was constructed and tried. It was found, however, that the central box in which the flies are placed was too large and that the flies only accidentally entered the two tubes at the sides. Moreover, it appeared from other tests that the flies do not easily enter into a tube which has no ventilation except at its entrance.

The apparatus shown in Fig. 1 was constructed after several trials and is believed to satisfy the requirements of flies such as blowflies, houseflies and others, which are sufficiently active. The apparatus has also been tried for mosquitoes, but these have the habit of settling down in a suitable spot and do not move about sufficiently to give the desired result.

The apparatus consists of a box A, measuring about 35 by 15 by 15 cm. The entrance is guarded by a gauze " sleeve ", the sides consist of wire screen and the exit is controlled by the sliding door B. This leads into a small box C, measuring 12 by 12 by 12 cm. Its top and front are made of glass, the rest being metal (or wood), and each side contains a circular opening of 7 cm. diameter surrounded on the outside by a short projecting collar. Further are required several tubes, which can most conveniently be made of celluloid, measuring 60 cm. in length with a diameter of 7 cm. They must fit well into the lateral openings of box C. Each tube is provided with four longitudinal openings or " windows " which are covered with gauze cloth and these must be equally large and evenly spaced in all the tubes. These windows provide ventilation and prevent the smell of the repellent tested from spreading through the whole apparatus. When the apparatus is used the windows

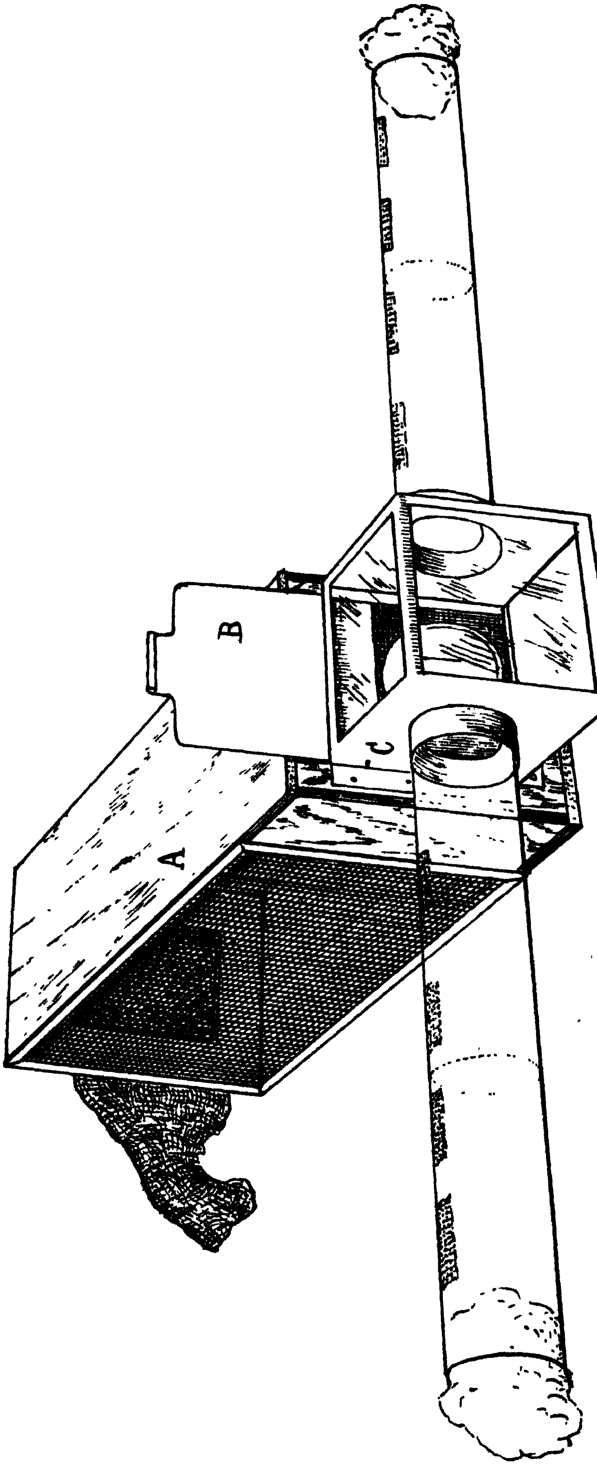

FIGURE I.

should therefore face upwards. The top and front of box C were at first made of wire gauze, but it was found that the flies would then not readily enter into the tubes.

The apparatus is set up as shown, the ends of the tubes being plugged with cotton wool and the box C facing towards the light. It is best to work in a large room where there is no draught and not too near a window, placing the apparatus in such a position that the light is distributed as evenly as possible over both the tubes. This light factor is very important. About 60-80 flies are placed into box A and they will soon pass through into C. The trapdoor B may now be closed, but as a rule the flies will not return into the darker box A and the open door provides further ventilation, keeping the smell of the repellent from entering the blank tube.

A blank count is first made, after allowing the flies a few minutes to get accustomed to the apparatus. Counts may be made every 30 or 60 seconds and 20 counts are made in all. Only the flies in the distal half of each tube should be counted, and for this purpose it is necessary to draw a line around the middle of each tube. It is convenient in the interval between counts to keep count of the flies in one tube so that at the correct moment attention can be given to the other to register the number there. When repellent has been introduced into one tube the flies in this tube should be watched in order to see how they react to the material when they come near it.

The preliminary blank count will show whether the distribution of light is sufficiently even on both sides. Necessary adjustments should be made. A drop of the repellent is now placed on to a plug of cotton wool and this is slipped into the place of a plug on one side. Beginning after a few minutes 20 counts are made. The plug with the repellent is replaced by a clean one and the flies are then chased back into C by pushing the plugs into the proximal ends of the two tubes; they are further brought into A by turning this side to the light and placing a dark cloth over C if necessary and then closing the door B. Fresh tubes are inserted, the repellent being now placed on the other side, and the flies are allowed to return. The apparatus should be replaced exactly in its previous position. Another 20 counts are made, fresh tubes again inserted and finally 20 blank counts complete the test. Instead of inserting fresh tubes for the second count with repellent the whole apparatus may simply be turned upside down, so that the repellent is now on the other side, but care must be taken to regain the exact previous position with reference to the source of light. The counts are finally summed up and, if necessary, corrections are made as indicated by the blank counts.

Tests with tagetes oil were made as follows:—

1. Flies: *Musca domestica, Lucilia sericata, Chrysomyia albiceps, Chrysomyia chloropyga* and *Sarcophaga haemorrhoidalis:* The blank counts were sufficiently even. Repellent, 68; empty tube, 200.

2. Flies: *Lucilia sericata, Chrysomyia chlorophyga:* Repellent, 55; empty side, 255.

3. Flies: *Lucilia sericata:* Repellent, 75; empty side, 216. In this case a few flies were apparently overcome by the tagetes oil and they remained in the end of the tube in a dazed condition during the rest of the count, being counted each time.

4. Flies: *Lucilia sericata:* Repellent, 65; empty side, 749.

5. Comparative test with oleum picis and *Lucilia sericata:* Repellent, 248; empty side, 347. The oleum picis does not show up well in this apparatus. Flies will even settle down on the plug carrying the material.

It appears therefore that the tagetes oil has definite repellent properties, and it has also been noticed that blowflies approaching a wound which has been treated with mixtures containing the oil will rapidly fly off without settling down.

C. TESTS WITH LARVICIDES.

It is not necessary here to recount all the tests made with different materials, most of which had been tested before by other workers. No other substance that could be used on a wound was found to be even nearly as effective as carbon tetrachloride and tetrachlorethylene, both known to be strong insecticides.

The tests were made as follows: *Lucilia sericata* eggs were collected by allowing the flies to lay on small pieces of liver. The larvae were allowed to develop in suitable surroundings until they began to migrate from the food material. These fully-developed third stage larvae are known to be the most resistant stage and were used in order to maintain this factor as constant as possible.

As a rule 20 larvae were placed in a suitable tube and the mixture to be tested was poured over them to a depth of about 2·5 cm. The tube was gently shaken and the period of reaction timed by means of a stopwatch. At the end of the time the mouth of the tube was covered with a piece of gauze cloth, the tube turned upside down and shaken so as to drain the fluid off quickly. The larvae were then transferred to tubes containing clean sand and these tubes were closed with gauze cloth. After allowing sufficient time for pupation and emergence of the flies and after the latter had died, the contents of each tube were sived out and the number of dead larvae, dead pupae and flies counted, as recorded below.

In order to ensure satisfactory contact between the material tested and the larvae it was decided to emulsify the volatile substances such as carbon tetrachloride, tetrachlorethylene and benzine. Oily mixtures, when applied to wounds, have the undesirable effect of softening the tissues and preventing the formation of a healthy scab. Watery emulsions dry off rapidly and only the less volatile portions remain on and around the wound. The mixtures used in the tests recorded below were not all satisfactory emulsions as will be discussed later.

For the sake of brevity the following abbreviations are used:— CCl_4 = Carbon tetrachloride, C_2Cl_4 = Tetrachlorethylene, T.ol. = Tagetes oil, G.ar. = gum arabic, Aq. = Water (distilled).

I.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
1. Benzine 10, T.ol. 10, G.ar. 5, Aq. 75.	1	1	2	12
2. ,, 10, ,, 10, ,, 5, ,, 75.	3	0	0	20
3. ,, 20, ,, 10. ,, 5, ,, 65.	1	18	1	1
4. ,, 20, ,, 10, ,, 5, lead acetate 3% 65............	3	20	0	0
5. ,, 20, G.ar. 5, Aq. 75........	3	18	2	0
6. C_2Cl_4 10, T.ol. 10, G.ar. 5, Aq. 75	3	18	0	2
7. Aq. 100 (42 larvae)..................	3	0	3	39

It is seen that 10 per cent. benzine even for 3 minutes is not sufficiently effective and compares rather unfavourably with tetrachlorethylene; 20 per cent. gave better results but was disappointing in subsequent tests. The lead acetate was added on account of its favourable effect on wounds and it did not break the gum arabic emulsion.

II.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
8. Benzine 20, G.ar. 5, Aq. 75...............	2	12	6	2
9. ,, 20, ,, 5, 3% lead scetate 75...	2	1	0	19
10. C_2Cl_4 20, ,, 5, Aq. 75...............	2	20	0	0
11. ,, 20, ,, 5, 3% lead acetate 75...	2	20	0	0
12. CCl_4 20, ,, 5, Aq. 75...............	2	19	1	0
13. ,, 20, ,, 3, 3% lead acetate 75...	2	12	6	2
14. T.ol. 10, ,, 5, Aq. 85...............	1	1	0	19
15. 3% lead acetate 100......................	2	0	0	20

In this case the lead acetate appears to have counteracted the effect of benzine and to a slight extent also that of carbon tetrachloride, but this is probably not the correct interpretation of the result—see 3 and 4 above and other tests below.

Tagetes oil has no appreciable larvicidal effect. It was decided to try woolgrease as emulsifier, since this would be particularly suitable for use on sheep. Emulsions with lead acetate were, however, not satisfactory and apparently did not bring about satisfactory contact between the insecticide and the larvae:—

III.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
16. C_2Cl_4 20, T.ol. 10, woolgrease 5, 3% lead acetate 65..................	2	5	0	15
17. ,, 20, T.ol. 10, G.ar. 5, 3% lead acetate 65	2	20	0	0
18. ,, 20, woolgrease 5, 3% lead acetate 75	2	0	2	18
19. Benzine 20, T.ol. 10, woolgrease 5, 3% lead acetate 65..................	2	0	1	19
20. ,, 20, T.ol. 10, G.ar. 5, 3% lead acetate 65........................	2	3	1	16
21. ,, 20, woolgrease 5, 3% lead acetate 75	2	0	0	20
22. Aq. 100..............................	2	0	0	20

Soap emulsions were now tried and it was found that the substances tested could very easily be emulsified as follows:—Benzine, CCl_4 or C_2Cl_4 50 cc.; Tagetes oil, 25 c.c.—A. Soft soap, 9 gm.; water to make 25 cc.—B. Add successive small quantities of A to B and shake. The thick emulsion is diluted with 150 cc. water, giving the following concentrations: CCl_4, 20 per cent.; Tagetes oil, 10 per cent.; Soap, 3·6 per cent. In these tests 40 larvae were used in each case.

IV.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
23. C_2Cl_4 20, T.ol. 10, soap 3·6, Aq. ad 100...	1	5	0	35
24. ,, ,, ,, ,, ...	2	27	3	10
25. ,, ,, ,, ,, ...	3	24	6	10
26. CCl_4 20, ,, ,, ,, ...	1	10	7	23
27. ,, ,, ,, ,, ...	2	34	5	1
28. ,, ,, ,, ,, ...	3	39	1	0
29. Benzine 20, ,, ,, ,, ...	1	2	1	37
30. ,, ,, ,, ,, ...	2	0	3	37
31. ,, ,, ,, ,, ...	3	2	1	37
32. Water..............................	3	1	2	37

The above test showed, as had been noted before, that CCl_4 and C_2Cl_4 were more effective larvicides than benzine. Since the temperature of the sheep's body would probably have an influence on the reaction by increasing the respiration of the larvae, the above test was repeated with the modification that the emulsions were warmed in a water-bath to 28° C. before being poured on to the larvae. In each case 20 larvae were used.

V.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
33. C_2Cl_4 20, T.ol. 10, soap 3 6, Aq. ad. 100...	1	7	1	12
34. ,, ,, ,, ,, ...	2	19	1	0
35. ,, ,, ,, ,, ...	3	20	0	0
36. CCl_4 20, ,, ,, ,, ...	1	4	11	5
37. ,, ,, ,, ,, ...	2	20	0	0
38. ,, ,, ,, ,, ..	3	20	0	0
39. Benzine 20, ,, ,, ,, ..	1	1	1	18
40. ,, ,, ,, ,, ...	2	0	1	19
41. ,, ,, ,, ,, ...	3	2	1	17
42. Water...............................	3	0	0	20

With regard to the reactions of the larvae under the influence of these emulsions it was observed that CCl_4 stunned them almost immediately, while C_2Cl_4 appeared to irritate them strongly for a few seconds before they quietened down and became motionless. It was thought that this property of the latter drug might be favourable, since it would cause the maggots to crawl out of deep wounds before they were killed. Tests made on sheep, however, showed that C_2Cl_4 unfortunately irritates the wound and the animal to a similar degree and that it had to be discarded for this reason.

It was further found in testing various emulsions on sheep that the free alkali of soft soap was irritating and the soap emulsions had to be given up. Woolgrease was again tried and satisfactory emulsions could be made in the following way:—Weigh off into a suitable flask 60 gm. woolgrease; measure off (A) 200 c.c. CCl_4 + 50 c.c. Tagetes oil (it had been found that 5 per cent. of this oil is sufficient as a repellent in the mixture); measure off also (B) 700 c.c. water. Add about 20 cc. of A and 100 of B to the woolgrease and shake or stir to emulsify, then add more water and, if necessary, further small quantities of A up to 60 c.c., shake to emulsify and go on adding water until all of B has been incorporated. Then add the rest of A and shake. Owing to the fact that woolgrease contains small quantities of free acid, the emulsion will not keep well. A few drops of phenolphthalein are therefore added—about 10 drops to the above quantity—and the emulsion is neutralised by adding a small quantity of 10 per cent. NaOH solution. After a few days the pink colour of the indicator may disappear on account of the liberation of further small quantities of acid and alkali should be again added in sufficient quantity to neutralise, or sufficient may be added the first time to give a definite pink colour. Since different samples of woolgrease vary in acid content the amount necessary cannot be stated definitely, but 12 c.c. of 10 per cent. NaOH to a litre of emulsion may be an average quantity to go by.

The emulsion may also be made by leaving out the Tagetes oil at first and adding it later to the neutralised emulsion of woolgrease, CCl_4 and water.

Tests with the above emulsion gave the following results:—

VI.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
43. CCl_4 20, T.ol. 5, woolgrease 6, Aq.ad. 100 (25°C.)	1	20	0	0
44. ,, ,, ,, ,,	2	20	0	0
45. ,, ,, ,, ,,	3	20	0	0
46. ,, ,, ,, (30°C.)	1	20	0	0
47. ,, ,, ,, ,,	2	20	0	0
38. ,, ,, ,, ,,	3	20	0	0
49. Water..................................	3	0	0	20

The above result was so satisfactory that it was decided to repeat the test and also to try 10 per cent. CCl_4 in the same emulsion. In test VI, the room temperature had been 25° C., in test VII it was 21° C., and this was also the temperature of the emulsion (40 larvae used in each case):—

VII.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae	Flies.
50. CCl_4 10, T.ol. 5, woolgrease 6, Aq.ad. 100 (21°C.)	1	22	2	16
51. ,, ,, ,, ,,	2	22	9	9
52. ,, ,, ,, ,,	3	20	13	7
53. CCl_4 20, T.ol. 5, woolgrease 6, Aq.ad. 100 (21°C.)	½	39	1	0
54. ,, ,, ,, ,,	1	39	1	0
55. ,, ,, ,, ,,	2	36	4	0
56. ,, ,, ,, ,,	3	40	0	0
57. ,, ,, ,, (30°C.)	½	40	0	0
58. ,, ,, ,, ,,	1	40	0	0
59. ,, ,, ,, ,,	2	40	0	0
60. ,, ,, ,, ,,	3	40	0	0
61. Water..................................	3	0	17	23

CCl_4 10 per cent. is apparently not sufficient and it may be advisable to keep to the 20 per cent. emulsion. The fact that 17 pupae failed to emrge in the case of the control may reduce the value of the test to some extent. However, in Nos. 56-60 the larvae never moved after having been immersed but gradually turned black and dried out.

With regard to its larvicidal effect this emulsion may be considered as satisfactory.

D. TESTS ON SHEEP.

While the mixtures and emulsions described above were being tested on larvae, they were also tested on infested sheep, as already indicated, and the satisfactory larvicidal effect of carbon tetrachloride, as well as the fly repellent properties of Tagetes oil was clearly demonstrated.

The requirements of a good blowfly mixture are: (1) Strong larvicidal effect; (2) absence of irritation and interference with the healing of the wound; (3) prevention of re-infestation until the wound is healed.

The emulsion described above satisfies these requirements to a high degree. The larvicidal effect is eminently satisfactory. The carbon tetrachloride-woolgrease emulsion with Tagetes oil has very slight irritant properties, if any. Some sheep will be uneasy for a few moments after the application of the emulsion, but this passes off very quickly and there is no sign of irritation or interference with the healing of the wound. The writer has applied the emulsion to a fresh abrasion on his hand and noticed no irritation whatever.

The emulsion has been tested on a fairly large number of cases with moderate to very large wounds and in no case did re-infestation occur, except in one sheep which lay prostrate and was repeatedly wetted by rain. This case was re-infected five days after the first treatment. The emulsion breaks soon after it has been applied to the wound. The larvae are killed within a minute and the carbon tetrachloride and water evaporate fairly soon, the length of time required depending on the humidity and temperature of the atmosphere. The wool-grease and Tagetes oil settle down in the wool surrounding the wound and the smell of the oil is in evidence for 10-14 days. The wound is usually dry after 24 hours and heals rapidly.

Tests are now being conducted with this emulsion under different conditions with reference to climate, pasture, etc., and also on cattle subject to attack by the screw-worm (*Chrysomyia bezziana*. In one case a farmer treated a number of infested cattle with complete success, no re-infestation occurring. The results of other tests are not yet available but will be reported upon at a later date.

It has been found that the 5 per cent. Tagetes oil can be replaced by 10 per cent. Oleum picis, but cases of re-infestation have occurred when such an emulsion was used.

SUMMARY.

It is shown that the steam-distilled oil of the plant *Tagetes minima* has strong repellent properties for blowflies and that it is suitable for use in a blowfly dressing.

Carbon tetrachloride and tetrachlorethylene are excellent larvicides for use against blowfly maggots, but tetrachlorethylene is irritating on wounds. Both these drugs are distinctly more effective than benzine.

Emulsions of the abovementioned substances are suitable as blowfly dressings and woolgrease was found to be the most satisfactory emulsifier.

Particulars are given in regard to the preparation of a suitable emulsion.

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Section II.

Bacteriology.

MASON, J. H. ... ... The Toxin of *Clostridium chauvoei* ... 433

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The Toxin of Clostridium Chauvoei.

By J. H. MASON, Section of Bacteriology, Onderstepoort.

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INTRODUCTION.

Cl. chauvoei is an anomalous member of the group of pathogenic spore-bearing anaerobes. Whilst small amounts (0·1 c.c.) of living cultures, inoculated subcutaneously or intramuscularly into susceptible animals (bovines, sheep, guinea-pigs) may produce gas gangrene and death within 24 hours, yet it requires a large dose (5 to 20 c.c.) of sterile filtrate administered intraperitoneally to guinea-pigs to kill them. One may call such a filtrate " toxin ", but it is hardly to be compared with the exotoxin of *Cl. welchii* and certainly not with that of *Cl. tetani*. Leclainche and Vällée (1900[1]) record that Martin's broth cultures are toxic after 48 hours' incubation and that toxicity increases up to 15 days. Of a filtrate of a 5-day culture, 5 c.c. to 6 c.c. killed a guinea pig in 12 hours, when administered intraperitoneally. They note that the toxin was heat stable. Kelser (1918) states that 1·0 c.c. to 1·5 c.c. of a filtrate of a 10 to 12 days' Martin's broth culture was lethal to guinea-pigs. Kojima (1923[1],[2]) shows that the filtrates of young (18 to 36 hours) cultures in Martin's broth plus 0·1 to 0·2 per cent. glucose and fresh guinea-pig muscle pieces were toxic for mice (intravenous injection) in doses ranging from 0·1 c.c. to 0·7 c.c. The toxin had an acute action, producing symptoms and death in from 2 to 3 minutes up to an hour or two. This toxin (or another constituent of the toxic filtrate) was haemolytic. Both were thermo-labile, resisted storage badly, and were neutralizable by *Cl. chauvoei* antiserum. Eichorn (1918) states that filtrates possess toxic properties and Leclainche and Vallée (1923) record that from the 24th hour, *Cl. chauvoei* secretes a toxin capable of killing, immediately, various species of animals. Basset (1925[1]) shows that the filtrate of serum liver broth cultures kills guinea-pigs if 15 c.c. to 20 c.c. are given intraperitoneally. The lethal properties are practically the same whether the filtrate is fresh, 2 to 4 months old or preserved in the light or in the dark. Scott (1925) and Viljoen and Scheuber (1926) were unable to demonstrate a toxin in *Cl. chauvoei* filtrates. Zeller (1925) used filtrates from three weeks' old peptone liver broth (plus liver pieces) cultures; 10 to 20 c.c. given subcutaneously or intraperitoneally to guinea-pigs did not kill them and swellings only were produced on the subcutaneous injection of 25 c.c. and 50 c.c. into sheep and 100 c.c. into an ox. The work of Grassberger and Schattenfroth (1908[1]) has not been confirmed. They were able to demonstrate a most powerful toxin in filtrates but there would appear to be little doubt that a contaminating toxigenic anaerobe was responsible for this. This view is supported by their own results (1908[2]) in which they show that guinea-pigs immune to the toxin were not immune to the inoculation of virulent black quarter material (e.g. muscle oedema fluid). There is little doubt that St. Ivanič's (1922) cultures were not pure. Of 8 strains investigated, 6 produced a powerful toxin, as little as 0·005 c.c. of filtrate killing rabbits when injected intravenously. The " oedema " serum of the Vienna Serotherapeutic Institute was able to neutralize 10 mouse lethal doses of toxin in a dose of 0·001 c.c. This serum had no neutralizing effect on the cultures of the two non-toxigenic strains. Kerrin (1934) demonstrated toxin and haemolysin in filtrates of buffered tryptic digest broth cultures. The lysin could haemolyse sheep red cells in a dose of 0·0025 c.c. to 0·005 c.c. Relatively small amounts (0·0025 c.c.–0·05 c.c.) of the sera of man, the ox, the sheep and the horse neutralized 10 minimal haemolytic doses, whilst 1·0 c.c. of the sera of normal guinea-pigs, rabbits and swine did not have this effect. The minimal lethal dose of the toxin (intravenous injection) was from 0·025 c.c. to 0·5 c.c. for mice and 0·5 c.c. for guinea-pigs; 3·0 c.c. did not kill a rabbit. The sera of man, horse, rabbit and guinea pig had a neutralizing effect on the toxin. The effect of the toxin was very quick. Heating the filtrate at 52° C. for 5 to 10 minutes destroyed the lysin and the toxin; however, both resisted storage fairly well.

The writer, in 1930 and 1931, at the Wellcome Physiological Research Laboratories, Beckenham, was able to demonstrate a weak toxin and lysin in the filtrates of young (18 to 24 hours) cultures of *Cl. chauvoei* (unpublished work). The results were similar to those noted above, viz., the intravenous injection into mice of from 0·1 c.c. to 1·0 c.c. of filtrate killed them with a very short incubation period (few seconds to half-an-hour) and from 2·0 c.c. to 5·0 c.c. was necessary to produce death in guinea-pigs. The lysin also was weak, the smallest haemolysing dose for sheep cells being about 0·025 c.c. but usually 0·1 c.c. was necessary. Toxin-antitoxin neutralization tests were unsatisfactory, owing to the small number of killing doses that could be used per test dose, often only one killing dose, and owing to the rapidity with which the toxin killed the animal. However, the experiments showed that an antitoxin made in a rabbit had more protective power than normal rabbit serum and the impression was gained that *Cl. septicum* antitoxin had some neutralizing effect. Unfortunately no definite conclusion could be drawn, as antitoxins made in horses against the toxins of *Cl. welchii*, *Cl. histolyticum* and *Cl. tetani* and the sera of some normal horses, bovines, goats and sheep sometimes neutralized one or two killing doses.

On the writer's arrival at Onderstepoort, the subject was taken up afresh, it being felt that if a " useable " toxin could be obtained, the disease, black quarter, could more easily be studied.

METHODS.

Cultures Used.

Cl. chauvoei.

Strain 64.—Isolated from a muscle of a heifer dead from black quarter in Waterberg (Transvaal) in 1929. This strain has been and is being used for the routine production of black quarter vaccine (anaculture) by the writer's colleague, Dr. J. R. Scheuber.

Strain D.—Brought from England by writer. Originally isolated from the muscle of a bovine, which died of black quarter in Europe.

Strain R. 77.—Isolated by the writer in 1934 from a piece of muscle of a bovine which died of black quarter in the Transvaal.

Strain L.—A strain supplied by Mr. D. A. Lawrence, Director of Veterinary Research, Southern Rhodesia. Mr. Lawrence informed the writer that this strain was being used for vaccine production and was relatively non-pathogenic for guinea-pigs and sheep. The writer was able to confirm this—2·0 c.c. to 5·0 c.c. of a 24-hour meat broth culture injected, intramuscularly, into a guinea-pig produced a swelling but not death.

Cl. septicum.

Strain K.F.—Brought from overseas by the writer, origin unknown. It produced a powerful toxin and had been used at the Wellcome Laboratories for the production of antitoxin.

PURITY OF CULTURES.

Cl. chauvoei 64 and *Cl. septicum* K.F. were " single-celled " by the method described by Mason (1936[2]). The remaining cultures were repeatedly plated and typical colonies picked. As guinea-pig passage cultures of *Cl. chauvoei* were repeatedly used, frequent recourse was had to surface purity and to fermentation tests. Throughout the work, candled or pulped filtrate was injected intravenously into mice, as a check on the presence of, for example, the toxins of *Cl. septicum* or *Cl. oedematiens*. Beyond, very occasionally, the presence of a staphylococcus (when the culture was discarded), no indication was got that the various cultures were other than pure. The fermentation reactions of the various strains were as follows (1 per cent. " sugar " in 1 per cent. peptone water plus 5 per cent. sterile sheep serum, 14 day's incubation in a McIntosh's and Fildes' jar at 37° C.):—

All four chauvoei strains fermented with acid formation and usually with the production of gas, saccharose, galactose, glucose, lactose, laevulose, maltose; in arabinose, a " trace acid " reaction was got; salicine, sorbite, mannite, dulcite, raffinose, rhamnose and inuline were not attacked. In litmus milk (plus 5 per cent. sheep serum) a soft clot and acid was produced Gelatine was liquified but Loeffler's serum medium and inspissated horse serum were not attacked.

The *Cl. septicum* strain (K.F.) produced acid and gas in galactose, glucose, lactose, laevulose, maltose, salicine, dextrine (±) and inosite. It was without action on saccharose, adonite, dulcite, raffinose, sorbite and mannite. An acid clot was formed in litmus milk, gelatine was liquified but neither Loeffler's medium nor solid serum was attacked.

The morphological, cultural and pathogenic peculiarities of the four chauvoei strains and the one septicum strain were those accepted as typical for these bacteria. K.F. produced a powerful exotoxin after 24–36 hours' incubation (M.L.D. for mouse i.v. between 0·005 c.c. and 0·05 c.c.) whereas, as will be shown in detail later, none of the chauvoei cultures produced a toxin the M.L.D. of which for a mouse was less than 0·1 c.c.

MEDIA.

Horse flesh meat broth (meat broth).—This was a slight modification of Robertson's (1916) medium. The preparation is given in the article by Mason and Scheuber (1936). In all instances, about half the volume of medium in the tube or flask was meat particles. Preparatory to inoculation, the pH of the medium was adjusted to 7·8 with NaOH, and then boiled for from 10 minutes (tubes of 10 c.c. capacity) to 2 hours (500 to 2,000 c.c. flasks) and cooled rapidly.

Pope's "straight line digest" medium.—[Pope and Smith (1932).]

Hartley's digest medium (1922).

Viljoen's and Scheuber's medium 1926 (see Mason and Scheuber, 1936, for preparation).

Von Hibler's medium (1908).

Colebrook's digest liver medium [see Mason and Scheuber (1936) for preparation].

As with meat broth, the pH of all meat-particle-containing media was adjusted to 7·8, and boiled and cooled just prior to inoculation. (This does not apply to volumes of 10 or 20 litres: the pH sometimes was and at other times was not altered but owing to the bulk, boiling was not resorted to.)

Surface cultures were obtained on horse flesh infusion peptone agar, plus 10 per cent. of a mixture of equal parts of sheep serum, sheep haemolysed red cells and a saline extract of guinea pig liver (see Mason 1934 and 1936^2).

Boiled bacilli (Henderson 1932). It was found that dense suspensions of *Cl. chauvoei* could be obtained by growing the germ in meat broth for 18–24 hours, pulping the culture, discarding the meat and mushing up the pulp, containing the bacteria, in a small quantity of water. One could then express a considerable portion of the bacteria-containing fluid with a press and the organisms could then be washed and spun out in a centrifuge. The dense suspension, so obtained, could then be treated as described by Henderson.

Filter candles.—The Berkefeld N variety was used throughout.

Animals: Mice.—These were supplied by one dealer and weighed between 15 and 18 grammes.

Guinea pigs.—White or predominantly white animals were used and weighed between 250 and 300 grammes. A barium sulphide depilatory was used to remove the hair, for the intradermic titration of toxin.

Sheep.—These were of the Merino breed, varied in weight from 18 Kg. to 30 Kg. and had usually passed through an anthrax and/or blue tongue experiment. Intradermic titration of toxin was carried out on the bare portion of skin of the inner side of the hind leg.

EXPERIMENTAL.

PART 1.

THE DEMONSTRATION AND PRODUCTION OF A TOXIN.

Preliminary experiments.—In a series of preliminary experiments an attempt was made to demonstrate a toxic action of the supernatant fluid (spun) or of the pulped or candled filtrate of a 6 to 18 hours' *Cl. chauvoei* culture (meat broth plus 5 per cent. haemolysed cells and serum). From 10 c.c. to 500 c.c. of medium was used and particular attention was paid to a thorough boiling to expel dissolved air. In some instances the medium was boiled, cooled, inoculated and then exhausted in an air-tight jar until bubbles ceased to appear. The jar was then washed out 2 or 3 times with hydrogen and finally filled with this gas and incubated. In one experiment, the following substances were added to the medium, prior to inoculation—chalk (excess), saponin (1 in 40 of a 0·4 per cent. solution), cystein (0·2 per cent.) and ferrous sulphate (1 in 40 of a 0·4 per cent. solution). To a number of filtrates, sodium hydrosulphite ($Na_2S_2O_4$) was added to make a concentration of 0·01–0·1 percent., and exhaustion carried out for 2–3 hours. The inoculum in all instances was the heart blood and/or liver of a guinea-pig just dead from an intramuscular inoculation of *Cl. chauvoei* or a meat broth culture once removed from a guinea-pig passage.

The results of the use of these various products (eleven in all) were as follows—guinea-pigs died after receiving 0·5 c.c.-2·0 c.c. intravenously (cardiac puncture). Symptoms usually developed in 2 to 10 minutes, the animal being distressed and breathing in a pumping fashion. It might then die within a further 5 to 30 minutes or might apparently recover to die within 24 hours. Some showed this distress but recovered and remained well. The most striking post-mortem appearance was the presence of petechiae or suggilations in the lungs; in some cases the lungs were a haemorrhagic mass. The serum from a sheep which had received anaculture and then living culture and survived, neutralized the toxic effect of the filtrate whereas normal sheep serum did not do so (filtrate and serum mixed, left for 1 hour at room temperature and injected). However, the antiserum was not able to prevent the appearance of the initial symptoms of distress.

Mice usually survived the injection of 0·5 c.c. intravenously As a rule, early symptoms of distress appeared, to pass off in from 5 to 30 minutes.

Two sheep, one normal and one immune, received intravenously, 200 c.c. of a pulped filtrate which killed guinea pigs in a dose of 1·5 c.c. No symptoms or disturbances were observed.

No indication was got that any one method of toxin production was better than another (period of growth, exhaustion, reduction, etc.). These preliminary experiments indicated that there was a very weak toxic material in chauvoei filtrates, but that, unless it could be concentrated, no useful work could be carried out. Before attempting concentration, the effect of intradermic injection in guinea pigs was ascertained.

Experiment 1.*

Strain 64 was grown in 3 × 40 c.c. flasks of meat broth plus 5 per cent. serum and haemolysed cells in a MacIntosh's and Fildes jar. After 18, 48 and 72 hours' incubation a tube was removed and filtrate injected intradermically into guinea-pigs. In addition the supernatant of the spun material or the candled sample of each day's filtrate was reduced with $Na_2S_2O_4$ and exhausted and its intradermic effect ascertained.

The one and the two day filtrates produced small red-blue areas on the skin, apparent after 2–3 hours; the three day filtrate formed a very pale red flush. Anti-chauvoei sheep serum neutralized this reaction, whilst normal sheep serum had little if any effect.

Experiment 2.

As experiment 1 indicated that 18-hour filtrates contained a product which caused a reaction on intradermic injection, an attempt was made to concentrate this material by drying. Strain 64 was grown in meat broth plus serum and haemolysed cells for 18 hours, in an air-exhausted jar. Of the candled filtrate, 0·2 c.c. produced a faint red area on the skin. A dry powder was made by drying down 30 c.c. over H_2SO_4; 30 mg. caused an intense blue mark, only slightly affected by chauvoei antiserum but not by normal serum. The same amount gave rise to a small red reaction in a normal sheep (36859) and to no reaction in an immune sheep (37405). Further experience indicated that part of the reaction in guinea-pigs was due to non-specific material, in great measure, to NaCl. Some batches of dried and powdered broth caused quickly-appearing bloody reactions. However, these tended to disappear quickly and the area was, as a rule, only with difficulty located after 24 hours.

Experiment 3.

The experiments collected under this heading were continuations of experiment 2. Strain 64 was cultured for 18 hours in meat broth plus serum and haemolysed cells and pulped or candled filtrates were either dried down or saturated with ammonium sulphate (about 60 gm. per 100 c.c.) and the precipitate dried. Such powders, in a dose of from 10 to 30 mg., produced intradermic reactions in guinea-pigs and sheep. Chauvoei immune sheep† either did not react or only very slightly. Chauvoei antiserum reduced the size and the intensity of the reactions (blue-red spots) but did not annul them entirely. Thus, there was reason to hope that the toxin could be further concentrated. With this end in view 2 × 2 litre quantities of meat broth plus serum and haemolysed cells were inoculated direct from a guinea pig, incubated for 18 hours and pulped. The wet precipitate produced by saturating with ammonium sulphate was dialysed, in parchment paper, for four days against distilled water. This was carried out at 10° C. to minimise any bacterial growth that might occur. The precipitate from 1 litre was placed in the paper, water added and this dialysed against about 16–20 litres of water, which was changed twice daily. The final outside fluid gave no precipitate or only the faintest opalescence when barium chloride was added. (Later on it was found advantageous to carry out the first 4–6 hours dialysis again running tap water). A copious, white, rather gelatinous precipitate formed in the bag. This was spun off and the supernatant dried down, first with fans blowing over shallow layers, and then when nearly dry, in an exhausted desiccator over H_2SO_4. Some experiments with the dry toxin so formed (Toxin 19) will now be detailed.

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* It is to be assumed throughout, unless otherwise stated, that the pH of the medium was rectified and the medium boiled just prior to inoculation.

Further it is to be assumed that the inoculum was either the heart blood and or liver of a guinea pig just dead from black quarter or a culture not far removed from a passage. When the statement is made that the inoculum was the liver of a guinea pig, it refers to the liver of a guinea pig just dead of an experimental infection.

† Unless otherwise stated, a chauvoei immune sheep means one which has received anaculture and living culture.

Toxin 19.—The dry powder was not fully soluble in saline, distilled water or broth, a white, rather sticky precipitate being got after spinning. The pH of the supernatant was 5·5–5·6; on adjusting the pH to 7·4–7·6 nearly all the deposit went into solution. However as this did not render the material more toxic, it was evident that the deposit was inactive, so that, as a routine, the powder was stirred up in the saline, spun and the supernatant used. After several trials, it was found that 100 mg. in 1·0 c.c. gave a solution that was mobile enough for the purposes required.*

Boiling the toxin for half-an-hour produced a considerable coagulum; heating at 60° C. for half-an-hour caused opalescence; 0·1 c.c. of neither produced a recognizable intradermic reaction in guinea pigs.

Minimum Reacting Dose (*M.R.D.*) *in Guinea-pig.*—This was found to be in the region of 0·01 c.c. A dose of 0·1 c.c. produced a red flush in half-an-hour, increasing in intensity after 2 hours and being at a maximum in 4–6 hours. After 24 hours, there was usually a tendency to spread with a less intense colouration. Doses around the M.R.D. came up more slowly, being apparent in 2–6 hours and recognizable as small reddish-blue marks after 24 hours.

Toxicity on Intravenous (*i.v.*) *Injection.*—A dose of 0·25 c.c. did not kill a mouse and 0·5 c.c. was non-lethal for a 150 gm. guinea-pig.

Reactions in Sheep.—Two immune sheep and one normal sheep received 0·25 c.c. intrademically. In 6 hours, the reaction in the normal animal was manifesting itself and in 24 hours was definite. This consisted of a very roughly circular red-blue area about 20 mm. in diameter, of a more intense hue in the centre. After 48 hours it had faded somewhat and was only a pale flush after 72 hours. No reactions appeared in the two immune sheep.

Specificity.—Ten M.R.D. (0·1 c.c.) were neutralized by 0·005 c.c. (not by 0·0025 c.c.) of the serum of one immune sheep, by 0·1 c.c. (not by 0·05 c.c.) of that of another but not by 0·1 c.c. of the sera of two normal sheep. (Using other toxins, it will be shown that 0·1 c.c. of normal sheep serum does not neutralize one sure M.R.D.).

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*Throughout the work with different toxins 100 mg. of dry toxin was dissolved in 1·0 c.c. saline. Therefore, the amounts of toxin injected will be given in c.c. and not in mg.

Comment on Toxin 19.—It appeared reasonably certain that a toxin had been demonstrated in a *Cl. chauvoei* filtrate, and that the intradermic injection into guinea-pigs was a satisfactory method of demonstrating it. One disadvantage was the rapidity with which the reaction faded. Often after two hours, what looked like a forerunner of a strong reaction was visible—a definite dark red area of about 10–15 mm. in diameter—to fade to a faint red flush within 18 hours. The overcoming of this difficulty and the investigation of other methods of concentration was tackled next.

ATTEMPTS TO CONCENTRATE TOXIN.

Experiment 4.

(*a*) *Effect of Dialysing in Parchment Paper and in Cellophane.* Strain 64 was grown for 18 hours in meat broth plus 10 per cent. of horse serum* and 2 per cent. of guinea-pig liver extract. The pulped filtrate was precipitated with ammonium sulphate and the precipitate dialysed against distilled water for 3 days in parchment paper and in cellophane (commercial variety obtained in South Africa, grade number unobtainable). Both inside fluids were treated as described for Toxin 19. The M.R.D. and Lr of each was approximately the same, with, if anything, the advantage to the "cellophane" toxin (Toxin 35).

(*b*) *The Effect of Dialysing for different lengths of time.*—Strain 64 (direct from a guinea-pig liver) was grown for 18 hours in 500 c.c. flasks of meat broth plus 10 per cent. horse serum. The precipitate produced by saturating the pulped filtrate with ammonium sulphate was dialysed in cellophane for different periods, and the inside fluid dried down after removing insoluble material. The M.R.D. and in some cases the Lr was then established (table 1).

TABLE 1.

The effect of Dialysis on Cl. chauvoei *toxin (Toxin 34).*

Dialysis.	M.R.D. (c.c.).	Lr.
Nil†	?0·025	
3 hours	>0·1	
21 hours	0·025	
48 hours	0·005–0·01	0·0025
96 hours	0·01–0·025	0·01
144 hours	?0·1	
288 hours	?0·1	

Similar tests carried out with other brews of toxin confirmed, in large measure, the results of table 1, viz. that a 3 to 5-days' dialysis with removal of insoluble material produced the best results. The questionable reaction got with the non-dialysed precipitate is to be attributed to the high concentration of ammonium sulphate.

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* The serum, taken with aseptic precautions, was incubated at 37°C for 48 hours in the presence of 0·1 per cent. formalin prior to its addition to the medium.

† "Nil" means that the crude dried ammonium sulphate precipitate was used. The figures under Lr mean that those amounts in c.c. of a certain antiserum neutralized 0·1 c.c. of toxin, whilst 25 per cent. less antitoxin did not do so. The sign "—" indicates no test.

(*c*) *The Use of Media other than Meat Broth.*—The details of each individual experiment need not be recorded. The following media were used, usually in 500–1,000 c.c. amounts, the inoculum being guinea-pig liver or a culture once removed from it, and the incubation being 18 hours at 37° C.—Hartley's digest broth (plus meat particles and sheep serum and haemolysed cells), Pope's digest broth (plus meat particles and sheep serum and haemolysed cells), Colebrooke's liver digest broth mixed with horse flesh broth and strongly buffered (mixed 1 to 9, 1 to 5, 1 to 1 and 2 to 1, no meat particles) and Viljoen's and Scheuber's medium. Under this heading may be included experiments comparing the toxin-producing power of large volumes (10–20 litres) and small volumes (500 c.c.) of meat broth plus 5 per cent. horse serum, meat broth plus 5–10 per cent. horse serum, 5 per cent. sheep serum and haemolysed cells and guinea-pig liver extract (the saline extract of from half to one liver, added to 1,000 c.c. of medium), meat broth plus 5 per cent. horse serum when the peptone was added right at the start of the preparation of the medium and when it was added in the usual way after the preparation of the muscle extract and meat broth plus 0·25 per cent. glucose.

It can be stated definitely that Viljoen's and Scheuber's medium and large volumes of meat broth gave poor results. It is difficult to submit an opinion on the results with the use of the other media, because sometimes the one and at other times the other gave indications of producing the best toxin. At one time it was thought that the addition of as much as 10 per cent. of horse plasma was very advantageous. Although this was undoubtedly so, there was the disadvantage that only about half of the final dry powder was soluble in water or saline. After repeated comparative tests, meat broth (meat particles about one half by volume) plus 2 to 3 per cent of guinea-pig liver extract (or, if the inoculum was a guinea-pig liver, about a half liver to 500–1,000 c.c. of medium) was adopted as yielding good toxin, with a final powder of which a considerable percentage was soluble.

(*d*) *The effect of the inoculum.*—When the inoculum was a portion of the liver of a guinea-pig killed by the intramuscular injection of culture (or of a culture not more than one meat broth tube removed from it) more potent toxin (in M.R.D.) was obtained than when a culture distant from an animal was used. The following brief summaries of two experiments illustrate this:—

1. (*a*) *Strain* 64: 6 meat broth cultures removed from a guinea-pig passage—grown 20 hours in 1 litre of meat broth plus sheep serum, haemolysed cells and guinea-pig liver extract. M.R.D. of dissolved powder, 0·05–0·1 c.c.
   (*b*) As (*a*) but inoculum direct from a guinea-pig liver. M.R.D. 0·01 c.c.
2. (*a*) Strain L as received from Mr. Lawrence (5·0 c.c. avirulent for a guinea-pig). Grown 36 hours in 1 litre of meat broth plus guinea-pig liver extract. M.R.D., 0·05–0·1 c.c.
   (*b*) As (*a*) but inoculum direct from a guinea-pig liver. M.R.D. 0·005–0·01 c.c.

(*e*) *The effect of the length of incubation.*—The three illustrative experiments to be noted were conducted with strains 64, L and R 77 respectively, in the medium and method of choice noted under (*c*). They were carried out when a means had been found of "stabilising" the intradermic reactions (see page 447 under "adrenalin"); with such a means a definite reaction could be elicited with pulped or candled filtrate. Flasks of 500 c.c. capacity were used. Pulped filtrate and the same, precipitated with ammonium sulphate, dialysed for 4 days in cellophane and then dried were employed. Table 2 records the results.

The results given in Table 2 do not allow the drawing of definite conclusions, although the indication is that, with increasing length of incubation, the toxicity drops. This indication was strengthened by results obtained in media such as meat broth plus horse plasma, where a 20 hours' filtrate was more potent than one of 48 hours' incubation. It will be noted that, in conformity with diphtheria toxin, the antitoxin-binding-power of the toxins need not necessarily bear a close relation to the M.R.D. Although the M.R.D. of the 72 hours' precipitated toxin of 64 is 2½ to 5 times that of the 20 hours' toxin, the Lr has not altered. However, it is realised that the Lr titration was carried out at three-fold limits, so that one cannot be certain that there was no difference.

One difference between the dry toxins from young and old cultures was the relatively clear-cut intradermic reactions produced by the former compared with the somewhat indefinite flushes caused by the latter. Chiefly for this reason, toxins for routine purposes were prepared from 20 hours' cultures.

(*f*) *The Effect of the Strain.*—No indication was got that any one of the four *Cl. chauvoei* strains (64, D, R 77 or L) was a better toxin-producer than the other, when a virulent culture direct from a guinea-pig liver was used and when the medium was meat broth plus serum, haemolysed cells or liver extract and the incubation 20 hours.

(*g*) *The Precipitation of Toxin other than by Saturation with Ammonium Sulphate.*

1. Half saturation with ammonium sulphate: The toxicities of two dry powders obtained by saturating (60 gm. per 100 c.c.) and by half saturating a 20-hours' filtrate with ammonium sulphate with subsequent dialysis and drying were approximately the same.

2. Precipitation with potash alum: (*a*) The filtrate of a 20 hours' meat broth plus horse plasma culture of strain 64 was saturated with ammonium sulphate, the precipitate dialysed for 6 days and the inside fluid spun. Some of the clear supernatant was dried down and to another lot at pH 5·7 enough alum was added to make a 1·5 per cent. concentration. After washing the alum precipitate twice in distilled water, it was dissolved in 2 per cent. sodium citrate and dialysed for 48 hours. The inside fluid was then spun and the supernatant dried down. The M.R.D. of the original dialysed ammonium sulphate precipitate was 0·01 c.c., and of the final alum product 0·1 c.c.

TABLE 2.

The Effect of the Length of Incubation on Toxin Production.

Incubation.	Toxin.											
	Strain 64.				Strain R 77.				Strain L.			
	Pulped.		Precipitated.		Pulped.		Precipitated.		Pulped.		Precipitated.	
	M.R.D.	Lr.	M.R.D.	Lr.	M.R.D.	Lr.	M.R.D.	Lr.	M.R.D.	Lr.	M.R.D.	Lr.
20 hours	0·1	0·0001– 0·0003	0·01	0·0003– 0·001	0·025	0·0006– 0·002	0·015	0·001– 0·0015	0·05– 0·1	0·001– 0·003	0·01	0·006– 0·008
48 hours	0·05– 0·1	0·0003– 0·001	0·01– 0·02	0·001– 0·003	0·05– 0·1	0·0006– 0·002	0·01– 0·015	0·001– 0·003	0·025	0·001– 0·003	0·01	0·006– 0·008
72 hours	0·05– 0·1	0·0001– 0·0003	0·025– 0·05	0·0003– 0·001								
96 hours									0·025– 0·05	0·0003– 0·001	0·015	0·001– 0·002
120 hours					0·025	0·0003– 0·001	0·01	0·003– 0·01				
168 hours									0·05	0·0003– 0·001	0·015– 0·02	0·002 0·004

(All figures=c.c. For the M.R.D. titrations, 100 mg. of dry toxin were dissolved in 1·0 c.c. of saline. Lr.=the first figure is that amount of antitoxin which did not neutralize 0·2 c.c. of pulped toxin or 0·1 c.c. of dissolved toxin and the second is that amount which did neutralize these amounts.)

(*b*) A 20 hours' filtrate was treated with enough alum to make a 1·5 per cent. concentration, and the precipitate dealt with as noted under (*a*). The M.R.D. was 0·025 c.c.

(*c*) Samples of a 20 hours' filtrate were treated with enough alum to make respectively a 1·0, 2·5, 5·0, and a 10·0 per cent. concentration and the precipitates dealt with as noted under (*a*). The smallest M.R.D. (1·0 per cent. alum) was 0·025 c.c.

(*d*) The dry powder of a dialysed ammonium sulphate precipitate was dissolved in saline, the pH adjusted to 5·6 and alum added (1·5 per cent. concentration). The precipitate was treated in the manner noted under (*a*). In addition, the supernatant of the alum precipitate was dialysed for 48 hours and then dried down. The M.R.D. were as follows—ammonium sulphate precipitate 0·005–0·01 c.c., alum precipitate 0·005–0·01 c.c., alum supernatant 0·007–0·015 c.c.

(3) Precipitation with Sodium Sulphate.—Strain 64 was grown for 20 hours in meat broth plus 5 per cent. horse serum. To the pulped filtrate 20 gm. of sodium sulphate per 100 c.c. was added and the precipitate dialysed for 4 days and the resultant fluid dried. To the supernatant of the first precipitate a further 20 gm. per 100 c.c. added, and the precipitate dialysed and the resultant fluid dried. The M.R.D. of the first precipitate was 0·03 c.c. and of the second 0·04–0·05 c.c.

(4) Precipitation with Acetic Acid.—The same filtrate as noted under (3) was used.

Enough acetic acid was added to bring the pH to 5·2 and the precipitate collected (1st precipitate). The supernatant was brought to pH 4·7–4·8, and the precipitate collected (2nd precipitate). The supernatant of this was brought to pH 4·5 and the precipitate collected (3rd precipitate). These three precipitates were dried down *in vacuo* and 100 mg. shaken up in saline (the pH of the suspension was brought to 7·8 with ammonia). A considerable amount did not go into solution; the M.R.D. of each supernatant was more than 0·1 c.c.

(5) Precipitation with Zinc Chloride: Strain 64.—The pulped filtrate of a 20 hours' meat broth plus liver extract culture of strain 64 was treated as follows. One sample was saturated with ammonium sulphate and the precipitate dialysed in the usual way: to another volume enough zinc chloride was added to make a 1 per cent. solution. The precipitate after two washings with distilled water was dissolved in 2 per cent. sodium citrate, dialysed for 4 days, and the inside fluid dried down.

The M.R.D. of both toxins was between 0·005 and 0·01 c.c.

(6) Precipitation with Acetone Saturated with Benzoic Acid.—Five grammes of toxin 19 were dissolved in 100 c.c. of distilled water and the solution clarified by spinning. To the chilled supernatant at pH 5·5, 5·0 c.c. of a saturated solution of benzoic acid in acetone was added. A copious white precipitate formed immediately. This was kept cool and washed 3 times with acetone (100 c.c. per washing). The final precipitate was dried *in vacuo*. The powder was white, impalpable and went into solution readily in saline of pH 8·0–8·2.

Intradermically, amounts of toxin between 0·05 and 0·1 c.c. produced quickly-appearing blue spots, which disappeared in a few hours.

(7) Precipitation with Alcohol and Ether.—On a number of occasions, the precipitates obtained by adding from 2 to 6 volumes of alcohol or of ether to a 20 hours' filtrate were dried down and titrated intradermically in guinea-pigs. None of the powders went into solution readily and their toxicities were low.

(*h*) *Adsorption on and Elution from Kaolin.*—To 20 c.c. of toxin 19 at pH 8·5 (dissolved in Universal Buffer*) enough kaolin to make a 10 per cent. suspension was added. The suspension was kept cool and shaken periodically for 5 hours. The kaolin was then spun out and suspended in 10 c.c. of Universal Buffer at pH 5·5. After thorough agitation, the kaolin was again spun off. No toxin could be demonstrated in the supernatant.

(*i*) *Effect of Freezing and Thawing Toxin.*—Salimbeni and Loiseau (1934) state that diphtheria toxin may be concentrated by freezing the fluid and collecting the bottom third, after thawing has taken place at room temperature. In such a test, carried out with one toxin (29) the M.R.D. of the original toxin, the top and the bottom third of the frozen and then thawed fluid was approximately the same.

Comment on Concentration of Toxin.

No definite indication was got that better concentration of toxin could be produced consistently by methods other than the ammonium sulphate-dialysis technique. One experiment might indicate that alum yielded the best product but repeat tests did not necessarily confirm this. For this reason, the ammonium sulphate method was adopted as routine.

Experiment 5.

Attempts to Obtain Clearer Intradermic Reactions.

As previously stated, the intradermic injection of toxin produced, when 5 to 10 M.R.D. were used, a reaction appearing as a tiny flush in 5 to 15 minutes, increasing in size and intensity up to 3 to 5 hours but fading out considerably in 18 hours. Thus, the determination of the M.R.D. and Lr was attended with some difficulty. One gained the impression that if the toxin could be fixed to one spot for a hour or two or if the tissue could be slightly damaged a more persisting and therefore more easily read reaction would be obtained.

(*a*) *Effect of Different Diluents* (Toxin 36).—This toxin was dissolved (100 mg. in 1·0 c.c.) in the following diluents, spun to remove any insoluble material and the supernatants tested–saline (0·85 per cent.) at pH 5·5, 7·4 and 8·0, 1·0 per cent. glucose (in distilled water) at pH 7·4 and 8, 2·0 per cent. starch (pH 8), 1·0, 2·5, 5·0 and 10·0 per cent. peptone (Witte) in distilled water at pH 7·4, distilled water, salt solutions (NaCl) of 1·0, 2·5, 5·0 and 10·0 per cent. concentrations, 10 per cent. serum (horse) broth and 10 per cent. guinea-pig serum in saline (pH 7–7·2). In most cases an M.R.D. and an Lr test was carried out. No definite indication was got that any one of these diluents at whatever pH gave reactions superior to those obtained when the toxin was dissolved in 0·85 per cent. salt solution of about pH 7–7·2. Quickly-appearing bloody flushes were produced when distilled water and the higher concentrations of salt and peptone were used, but, in these cases, the control fluid, itself, without toxin, produced a similar reaction.

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* British Drug Houses, London.

A small amount of insoluble deposit was obtained when toxin 36 was placed in saline, the final pH of the supernatant being about 6. By raising the pH to 7·6–8·0, nearly all of this deposit went into solution but without lowering the M.R.D. or intensifying the reaction.

(*b*) *The Effect of Incorporating Agar in the Toxin.*—When the toxin was dissolved in 0·1 to 1·0 per cent. nutrient agar, the reaction produced was much more definite and persistent than when saline was the diluent. However, a difficulty was experienced in carrying out a toxin-antitoxin titration. It was difficult and, at times, impossible to ensure a thorough mixing of the toxin and antiserum, when 0·25 per cent. agar was employed. For this reason, results varying from day to day were got, depending upon the degree of admixture.

THE EFFECT OF ADRENALIN.

As adrenalin produces a vasco-constriction, the possibility existed that, mixed with toxin, it would localise it in the injected area, and thus give it the opportunity of producing an easily recognizable reaction. Experiments proved the correctness of this supposition. The adrenalin used was that prepared by Gehe and Co. A. G. Dresden, solution 1/1,000. Of this, 0·1 c.c. produced a very extensive haemorrhagic area in a guinea-pig's skin in 18 hours, the animal dying in 48 hours. Dilutions of 1/10, 1/20, 1/30 in 0·3 c.c. saline nearly always caused spreading haemorrhagic reactions, the animal usually dying or having to be killed. A borderline dose was 0·3 c.c. of a 1/50 dilution; sometimes a dirty-red spreading flush was produced, particularly if the site of injection was near the loose skin of the belly. There was less risk of causing a reaction when the adrenalin was injected into the firmer skin over the ribs. Dilutions higher than 1/50 proved relatively safe and after considerable experimentation 0·3 c.c. of a 1/100 dilution was chosen as being suitable. The toxin was localized for a time sufficiently long to produce a reaction readable after 24 to 48 hours whilst the risk of the non-specific effect of the adrenalin was very slight. In a quite negligible number of occasions, the adrenalin did, *per se*, cause flushes, but very little experience was necessary to distinguish these from toxin reactions and further, a repeat test invariably cleared up any doubt. The practice was adhered to of storing the adrenalin in an amber coloured bottle in a refrigerator and discarding it if it became discoloured in any way or if any deposit was discernible.

The reaction produced by the toxin plus adrenalin will be described under " the actions of the toxin ".

The Effect of Removing Insoluble Material from the Toxin.—No toxin has been prepared which is fully soluble when added to saline at pH 7·0. The pH of the toxin in saline solution is between 5·5 and 6·5 depending on the toxin. By raising the pH of the toxin solution to about 8, most of the undissolved material dissolves but, as has previously been stated, without a corresponding reduction of the M.R.D. An experiment was conducted in which a dry toxin was dissolved in saline, the deposit being removed by centrifugation. The supernatant was dried and the powder again dissolved in saline, and this process repeated three times. On each occasion, a precipitate was obtained on attempting to dissolve the toxin, and further, the final supernatant (toxin dissolved 100 mg. to 1·0 c.c.) was less toxic than the original. Thus, it would appear that each drying denatured some protein and destroyed some toxin.

THE ACTIONS OF THE TOXIN.

1. *The Effect of Intradermic Injection.*

(These remarks apply when the toxin is dissolved in a 1/100 dilution of adrenalin and 0·3 c.c. is injected.)

When 5 to 10 M.R.D. are injected into a guinea-pig a flush usually appears within 5 to 15 minutes, to disappear in about half-an-hour. For a further one or two hours the site of injection is recognized by the paleness of the skin, the effect of the adrenalin. This gradually disappears and 2 to 4 hours after treatment a somewhat strippled pale red spot appears increasing in intensity up to 8 hours. After 12 to 24 hours, a typical reaction (caused by 5 M.R.D.) is very roughly circular, from 1·5 to 3·0 cm. in diameter, with an intense red centre and a rather mottled-red radiating periphery. In some instances, 5 to 10 reacting doses produce a small area of necrosis in the centre, but the reaction is, in the main, haemorrhagic in nature. Amounts of toxin, around the M.R.D., produce nothing recognizabe for 6 to 8 hours and are visible after 24 hours as small red flushes measuring 1 to 2 cm. in diameter. (See Fig. 1.)

As much as 10 guinea-pig reacting doses did not cause a lesion in rabbits.

The M.R.D. for the sheep was approximately that for the guinea-pig, the injections being made into the skin of the thigh. All the sheep used at Onderstepoort were docked so that the much thicker and tenser skin of the under surface of the tail could not be used. Experiments carried out by Dalling, Gordon and Mason at the Wellcome Physiological Research Laboratories proved that this was a most suitable site for the intradermic titration of *Cl. welchii*, Type B toxin (unpublished work), and the writer suggests that the same would hold for *Cl. chauvoei* toxin.

2. *Lethal: (Intravenous Injection).*

Guinea-pigs.—(Toxin 37 L. M.R.D. = 0·01 c.c.)

The toxin was dissolved in saline (100 mg. in 1·0 c.c.) the deposit removed, and the dose injected in a total volume of 1·0 c.c.

A dose of 0·5 c.c. often produced a few spasmodic movements, with quickened respirations, in a few minutes. However, these passed off, to be followed in from 2 to 3 hours by weakness and hurried breathing. The animal lay on its side, occasionally uttering a squeak. Death usually supervened in 4 to 6 hours, being preceded by spasms. The minimal lethal dose of this toxin was between 0·1 and 0·15 c.c.

Mouse.—Per gram body weight, this animal was much less susceptible than the guinea-pig, the M.L.D. being 0·1 c.c.

Rabbit.—Twenty guinea-pig fatal doses produced only transitory distress.

Sheep.—The fatal dose proved to be between 10 and 15 c.c. In about half-an-hour the respirations became markedly accelerated and, a thick ropy mucous discharge appeared at the nostrils. In 2 or 3 hours, the breathing was of the pumping variety, strings of mucus hung from the nose, and occasionally diarrhoea was present. Incoordination of movement and later inability to support the body supervened and death occurred in from 6 to 48 hours after the injection.

Post-mortem Appearances.—In the guinea-pig, mouse and sheep, these were entirely of a haemorrhagic nature. The lungs were always the seat of bloody effusions, sometimes only petechiae being present but at other times the lungs were bloody masses. On the pleura and peritoneum petechial spots to suggilations were seen. The heart was usually flabby, with petechiae or suggilations on the endocardium. extensive haemorrhages were present in the wall of the intestine, and in one sheep haemorrhages had occurred in the gut-wall from the abomasum to the rectum. Blood was usually noticeable in the lumen of the intestine. In one sheep, which died half-an-hour after receiving 2 M.L.D., about 1 litre of bloody fluid was present in the pleural cavity, the lungs also being full of blood. This animal obviously drowned in its own exudate.

3. *Haemolytic.*

In conducting the haemolytic test, toxin was added to 1·0 c.c. of a 2·5 per cent. suspension of thrice washed sheep red cells, the total volume made up to 2·5 c.c. with saline, the tubes incubated at 37° C. for 2 hours and, after standing at room temperature for a further 2 hours, were read as complete (C), nearly complete (N.C.), partial (P), trace (T) or negative (O) haemolysis. In table 3 are recorded the minimal haemolytic doses (M.H.D.) and Lhs of a number of toxins. The Lh was arrived at by mixing 0·5 c.c. of the toxin with decreasing amounts of antitoxin, allowing the mixtures to stand for one hour at room temperature, adding 1·0 c.c. of red cell suspension and after adjusting the volume to 2·5 c.c. with saline treating the tubes as described for the M.H.D. test. In these tests, the haemolytic power of the pulped culture and of this pulped filtrate after precipitation, dialysis, drying and re-solution (100 mg. in 1·0 c.c.) was established.

TABLE 3.

The Haemolytic Power of Cl. chauvoei *Toxin.*

Age of Toxin.	Pulped toxin.		Precipitated toxin.	
	M.H.D.	Lh.	M.H.D.	Lh.
	STRAIN L (V).			
20 hours	0·1	0·03–0·1	0·1	0·03–0·1
48 hours	0·1	0·03–0·1	0·05	>0·1
96 hours	0·25	0·03–0·1	0·1	?0·1
168 hours	0·25	<0·01	0·05	0·03–0·1
	STRAIN L (V) (another brew).			
36 hours	0·05	0·03–0·1	—	—
	STRAIN L (AV.).			
36 hours	0·05	0·03–0·1	—	—
	STRAIN D.			
20 hours	—	—	0·1	0·1–0·3
	STRAIN 64.			
20 hours	0·025	0·03–0·1	—	—
	STRAIN 64 (another brew).			
20 hours	1·0	N.D.	0·2	0·03–0·1
48 hours	0·25	0·03–0·1	0·2	0·1–0·3
72 hours	1·0	N.D.	0·25	0·1–0·3

(All figures = c.c., precipitated toxin dissolved 100 mg. in 1·0 c.c. saline; N.D. = not done; age of toxin = number of hours that culture was grown; in the Lh titrations, 0·5 c.c. of toxin was used; the first figure is that amount of a certain antitoxin which did not neutralize the lysin contained in this amount of toxin and the second figure is that amount which did neutralize it; the M.H.D. and Lh titrations were carried out at 100 per cent. limits; the M.H.D. was taken as that amount which produced a "partial" or "large trace" haemolysis).

It will be noticed that neither the original pulped nor the precipitated filtrates of *Cl. chauvoei* are markedly haemolytic, from 0·025 c.c. to 1·0 c.c. being required to cause partial lysis of sheep red cells. Further, large amounts of antitoxin are required to produce neutralization, much more than was necessary to neutralize that portion of the toxin causing skin reactions. For example, 0·008 c.c. of a certain antitoxin (38212) was required to neutralize a test dose (0·1 c.c. = 10 M.R.D.) of strain L precipitated toxin, whereas 0·1 c.c. was necessary for 0·5 c.c. (5 M.H.D.) in the haemolytic test. The sera of different animals (not immunized against *Cl. chauvoei*) had a definite neutralizing power. The sera of normal horses, cattle, sheep, goats, rabbits and guinea-pigs in a dose of 0·1 c.c. could neutralize 5 to 10 M.H.D. of 37 toxin. The indications were that cattle and guinea-pig sera were poorer in this respect than those of the horse. No indication was got that the antitoxins of *Cl. welchii*, Types A, B or D, *oedematiens*, *septicum*, *tetani* (all made in horses), *histolyticum* (rabbit) or *botulinum*, Types A, B, C, and D (goats) contained more antihaemolysin than the sera of the normal animals in which they were made. Later it will be shown that few normal sera have a neutralizing effect on the skin-reacting product of *Cl. chauvoei* toxin. Thus, it is reasonably certain that the lysin and the toxin are serologically distinct.

It is possible that culture medium plays a big part in lysin formation. This may explain the stronger material obtained by Kojima (1923) and Kerrin (1934). However, using Hartley's and Pope's digest media, strong lysins were not obtained by the writer.

(4) "*Aggressive*" *Action of Toxin.*

On subcutaneous injection of the toxin into guinea-pigs or sheep, an appearance similar to that caused by culture, but without gas formation, was produced. Without recourse to smear examination and culture, it would be difficult to distinguish the one condition from the other. With one toxin (37 P) the M.R.D. of which was 0·01 c.c., 0·5 c.c. produced death in a guinea-pig in from 36 to 48 hours, with a dark red oedema from the sternum to the pubis.

The experiment recorded in table 4 shows that the toxin was able to activate spores. A 5-day-old meat-broth culture of strain 64 was spun, the bacillary deposit washed twice in saline and then after reconstitution in saline to one quarter of the original volume was heated at 60° C. for half-an-hour. To different amounts of this suspension, toxin was added, the total volume made up to 2·0 c.c. with saline and injected intramuscularly into guinea-pigs and sheep.

TABLE 4.

The Activating Power of Toxin on Washed and Heated (60° C.) Cl. chauvoei *Spores. Mixture Injected i.m. into Thigh.*

Animal.	Spore suspension (c.c.).	Toxin (c.c.).	Result.
G.P. 1	1·0	None	Nil.
G.P. 2	0·5	0·1	L.L. ✓
G.P. 3	0·5	0·25	+ o/n.
G.P. 4	0·25	0·25	+ 2 d.
G.P. 5	0·25	0·1	+ 1 d.
G.P. 6	None	0·25	L.L. ✓
G.P. 7	None	0·1	Nil.
Sheep 1 (37518)	0·5	0·01	+ o/n.
Sheep 2 (34477)	0·5	0·05	+ 1 d.
Sheep 3 (39745)	1·0	None.	Nil.
Sheep 4 (40751)	None.	0·5	L.L. ✓
Sheep 5 (40390)	0·5	1·0	L. ✓
Sheep 6 (40492)	0·5	0·5	Sl.L. ✓

(G.P.=guinea pig; + =died; ✓ =lived; o/n= overnight; d=day(s); nil=no reaction; L=lame; Sl.L.=slightly lame; L.L.= lame, leg swollen and skin red. Sheep 1, 2, 3 and 4 were normal; sheep 5 and 6 were immune.)

The fact that immunized sheep (Nos. 5 and 6) were able to withstand much larger doses of toxin than normal sheep (Nos. 1 to 4) suggested a method of testing the immunity produced in sheep treated with anaculture. Toxin and a spore suspension were prepared and the smallest activating dose of toxin was ascertained in sheep and guinea-pigs. Between tests the spore suspension was held at about 2° C. Unfortunately the suspension deteriorated rapidly and one month after preparation about ten times more than was used in the first test was required (plus the original minimum activating dose of toxin) to cause the death of the experimental animal. As will be shown later, the dry toxin is stable for at least one year at room temperature.

THE SPECIFICITY OF THE TOXIN.

When methods had been devised for producing toxin regularly and for titrating it reasonably accurately, every batch made was titrated against an immune sheep's serum and the sera of one or more normal sheep. This involved the conducting of an Lr test (5 to 10 M.R.D. were titrated intradermically against decreasing amounts of antitoxin) and the demonstration that 0·1 c.c. of normal serum was incapable of neutralizing 1 to 2 M.R.D. Table 5 records the results of a series of M.R.D. and Lr tests with toxin 37 L and antitoxin 38212.

TABLE 5.

The Accuracy of the M.R.D. and Lr Tests (Toxin 37 L).

M.R.D.

Dose (c.c.).	Number of tests.	Results.		
		+	±	—
0 0025	6	0	1	5
0 005	6	2	3	1
0 01	12	9	3	0
0 015	6	5	1	0
0·02	6	6	0	0

LR.

(Test dose toxin=0 1 c.c.)

Dose Antitoxin (c c.).	Number of tests.	Result.			
			+	tr	
0 0025	2	2	0	0	0
0 003	3	3	0	0	0
0 0035	4	2	0	2	0
0 004	4	0	0	1	3
0 0045	4	0	0	0	4
0 005	3	0	0	0	3

(The toxin was dissolved in 1 : 100 adrenalin-saline (100 mg. in 1 0 etc.--degrees of reaction; readings taken after 24 hours.)

The results just given show that *Cl. chauvoei* toxin and antitoxin may be titrated with a considerable degree of accuracy by the guinea-pig intradermic method and in the writer's opinion the accuracy compares favourably with the intradermic titration of the toxins and antitoxins of *Cl. welchii*, Type B and of *Cl. septicum*. Further, results, almost superimposable on those in tabtle 5 (but with different dosage) could be tabulated for at least 5 other brews of toxin.

The results obtained in titrating 1 M.R.D. (0·01 c.c.) of 37 L toxin against 0·1 c.c. of normal sera and against the antitoxins of other sporulating anaerobes may be summarised as follows: --The sera of the following normal animals had no neutralizing effect— 4 horses, 10 sheep, 10 guinea-pigs, and 4 goats. The serum of a two-months-old calf and of two one-year-old bullocks was without effect but that of a 2-year-old bovine neutralized 4 but not 6 M.R.D. One normal rabbit's serum reduced greatly the reaction produced by

1 M.R.D. Using another toxin (36 M.R.D. = 0·015) these results were obtained with 4 other rabbits. The serum of rabbit (1) neutralized 1 to 1½ M.R.D., that of rabbit (2) 3 M.R.D., that of rabbit (3) 2 M.R.D. and that of rabbit (4) 5 to 6 M.R.D. The following antitoxins, prepared in horses, rabbits and goats had no neutralizing power—*Cl. welchii*, Types A, B and D, *oedematiens*, *histolyticum*, *tetani* and *botulinum*, Types A, B, C and D. It is interesting, in view of the results just given for normal rabbit serum, to note that the *histolyticum* antitoxin was prepared in a rabbit. The results with *Cl. septicum* antitoxin will be dealt with separately, because it was capable of neutralizing a considerable amount of toxin.

The Serological Relationship of the Toxins Produced by Four Different Strains.—Toxins were prepared from strains 64 (42), D (37 L), L (44 a) and R 77 (40) and antitoxins made in sheep and goats. In the case of 64 toxin, a sheep received first anaculture, subcutaneously, then living culture intramuscularly, followed by anaculture in increasing bi-weekly doses and finally an injection of about 100 fatal doses of culture intramuscularly. The other antitoxins were made in goats by injecting, subcutaneously, increasing doses of toxoid (formol-filtrate). In table 6, the neutralizing doses of the antitoxins against test doses of the four toxins are recorded.

TABLE 6.

Neutralization of Cl. chauvoei *Toxins, made from Four Different Strains, by Antitoxins prepared in Sheep and Goats.* (*Guinea-pig Intradermic Method.*)

Toxin.			Antitoxin (c.c.) (neutralizing dose).			
Strain.	M.R.D.	T.D.	D.	64	L.	R 77.
	c.c.	c.c.				
D (37 L)	0·01	0·1	0·03	0·0035	0·008	0·1
64 (42)	0·015–0·02	0·1	0·008	0·0012	0·003	0·04
L (44a)	0·01	0·1	0·06	0·008	0·02	0·16
R 77 (40)	0·015–0·02	0·1	0·012	0·0012	0·0025	0·02
			Ratios.			
			8	1	2	28
			7	1	2½	33
			7	1	2½	20
			10	1	2	16

(T.D.=test dose. Toxin and antitoxin mixed, left for one hour and injected; antitoxin levels varied by 20–30 per cent.)

The results given in table 6 show that, no matter which toxin was used, the antitoxins were placed in the same order of value. It will be noticed that the ratios for antitoxin R 77 vary somewhat. The possibility exists that this antitoxin was very non-avid, as some difficulty was experienced in obtaining a sharp end point. A splay of ± or trace reactions from 0·01 c.c. to 0·02 c.c. was often got, indicating a loose combination of toxin and antibody.

Under appropriate headings, results will be presented showing that the injection into guinea-pigs or sheep of filtrate or formol-filtrate rendered them refractory to the intravenous and intradermic administration of toxin or the intramuscular inoculation ot living culture and that the serum of such animals contained neutralizing antibodies. The ability of the serum of a bovine of over two years of age and of rabbits to neutralize 1 to 6 M.R.D. of toxin is in accord with the known insusceptibility of these animals to the natural disease (bovines) or to the inoculation of living culture (rabbit). However, it is recognised that the presence of antitoxin in the blood may not be the only factor in operation. The horse is relatively insusceptible to black quarter, yet the sera of a number of normal horses and of horses immunized against the toxins of other anaerobes had no neutralizing properties. Again, as will be shown later, a sheep may be rendered highly immune to culture (and probably to the natural disease) without there being demonstrable antitoxin in the blood stream.

Thus, one may conclude that the presence of *Cl. chauvoei* antitoxin in the blood of an animal is usually a strong indication of a stimulus with the corresponding toxin (the word " usually " is purposely employed, as it is difficult to conceive of rabbits and not guinea-pigs receiving the stimulus). Further, the toxins of two South African, one European and one Rhodesian strain of *Cl. chauvoei* are serologically indistinguishable.

Flocculation.—A number of attempts to titrate toxin and antitoxin by Ramon's (1922) method failed. After establishing the neutral point intradermically in guinea-pigs, a test was put up using 1·0 c.c. of toxin and amounts of antitoxin varying from 100 per cent. more than to 100 per cent. less than the determined neutralizing dose. The mixtures, at pH 7·0, 7·4 and 7·8, were heated at 37° C. and at 50° C. respectively for 5 hours and then left for 18 to 48 hours at 10° C. No flocculation visible through a ×8 hand lens was discernible.

THE STABILITY OF THE TOXIN.

Dry toxins have retained their original values (M.R.D. and Lr) for at least two years. However, in the dissolved state, the precipitated toxins appear to be no more stable than the liquid filtrates. In Table 7, the effects on toxicity of heating pulped filtrate pH 6·5 (Strain 64, toxin 47) and of allowing it to stand for different periods at 5° C. without preservative are recorded.

TABLE 7.

The Effect of Heat and of Storage on Toxin (Toxin 47).

	M.R.D.	Lr.
	(c.c.).	(c.c.).
Original	0·015	>0·004
1 day 5° C.	0·025	0·002
2 days 5° C.	0·025	N.D.
3 days 5° C.	?0 025	0·001
½ hour 60° C.	?0 1	0 0007
½ hour 75° C.	>0·1	<0 001
½ hour 95° C.	>0 2	<0·001

(For the Lr. titrations, 0 2 c.c. of toxin was titrated against antitoxin, the levels of which differed by 20–30 per cent. The figures given under Lr=the amounts of a certain antitoxin required to neutralize the test dose of toxin.)

The results given in Table 7 show that the toxin is thermolabile and resists storage badly even at a low temperature and in sealed-off tubes. This experiment was confirmed on many occasions when it was necessary to store toxin for 18 hours in case a repeat test was necessary. Very often the M.R.D. rose and the Lr fell several hundred per cent. in this short time.

PART II.

THE PRODUCTION OF IMMUNITY.

PRODUCTION OF IMMUNITY WITH PRECIPITATED TOXIN.

It was anticipated that a relatively small amount of precipitated toxin would produce immunity to the inoculation of a lethal dose of virulent culture, because, if the immunity was brought about only by the soluble toxin, then the injection of a few milligrams of the dry material was equivalent to the administration of one to several cubic centimetres of the liquid filtrate. However, as Henderson (1932) has shown in small animals and Mason and Scheuber (1936) in sheep, immunity to culture does not necessarily depend entirely on the presence of toxin or toxoid in the vaccine injected. Heat-killed bacilli alone produce a good immunity to culture.

Three sheep received, subcutaneously, 0·01 c.c., 0·1 c.c. and 1·0 c.c., respectively of a dissolved dry toxin ("S.C." M.R.D. = 0·015 c.c. to 0·02 c.c.). Three weeks later, one did not withstand 1 M.R.D. of culture, inoculated intramuscularly, the second died from 2½ M.L.D. and the third from 10 M.L.D. (Sheep 38837, 36878, 36856, and control sheep 37583, 37105.) One cannot say, from this result, if any immunity at all was produced, but it is definite that it was not of a high order.

From a number of guinea-pigs which had been used for the intradermic titration of toxin and antitoxin, four were set aside and three weeks later, all received, subcutaneously, 0·5 c.c. of a dry toxin ("26a", M.R.D. = 0·01 c.c.). Sixteen days after this injection 0·4 c.c. of culture was inoculated intramuscularly into all. Three survived, and one died within 36 hours; normal control guinea-pigs which received 0·1 c.c., 0·15 c.c., 0·2 c.c. and 0·4 c.c. of the same culture died in from 1 to 3 days.

In an intravenous titration, sheep 41010 received 1·5 gm. and sheep 41530 2 gm. of toxin 49. Both survived. Prior to the injection, 0·1 c.c. of neither serum neutralized 1 M.R.D. of toxin 37 L. Three weeks later, 41010 received 3 gm. of toxin 49 intravenously; and survived. Two weeks later, 0·02 c.c. of the serum of 41010 neutralized 10 M.R.D. of 37 L toxin and 0·1 c.c. of that of 41530 10 M.R.D. Both survived the intramuscular injection of 10 M.L.D. of culture.

Thus, it is shown that precipitated toxin can stimulate the formation of antitoxin, and in large doses can immunize sheep and guinea-pigs against culture.

PRODUCTION OF IMMUNITY WITH FORMOLIZED FILTRATE.

It is well known that filtrate or formol-filtrate is a satisfactory antigen. The literature bears witness that guinea-pigs, sheep and cattle may be solidly immunized against culture or the natural disease by the injection of the one or the other of these antigens. Therefore, no good purpose would be served by presenting data on this point. However, the results given in Table 8 do prove that sheep which have received formol-filtrate develop circulating antitoxin, resist the injection of culture and do not react when toxin is injected intradermically. Strain 64 was grown for 18 hours in meat broth plus guinea-pig liver extract and pulped. To the filtrate at pH 7·4 enough formalin was added to make a 0·3 per cent. concentration and the whole incubated at 37° C. for 48 hours. The details of the experiment are recorded in Table 8.

It will be observed that no sheep had demonstrable circulating antitoxin prior to the injection of the antigen but that 0·1 c.c. of serum was able to neutralize from 2 to more than 6 M.R.D. of a certain toxin (37 L) in from 13 to 25 days after the second injection of formol-toxoid. The M.R.D. test was not carried out prior to immunization but results in many other normal sheep showed that only a very occasional one was able to withstand 1 M.R.D. It will be seen that all 8 sheep tested did not react to the injection of 2 M.R.D. of toxin. Three of four sheep resisted 1 M.L.D. (i.v.) of toxin and two showed only minimal reactions after receiving 10 M.L.D. of culture intramuscularly.

TABLE 8.

Immunization of Sheep with Formol-toxoid (Formol-filtrate). Sheep received 5 c.c. on 12.2.25 and 10 c.c. on 5.3.35 (Experiment 48).

Sheep.	Number M.R.D. toxin neutralized by 0·1 c.c. serum.				Number M.R.D. toxin withstood by sheep.	Number M.L.D. (i.v.) toxin withstood by sheep.		Number M.L.D. (i.m.) culture withstood by sheep.
	11/2/35.	18/3/35.	27/3/35.	30/3/35.	27/3/35.	28/3/35.	30/3/35.	31/3/35.
39777	<1			>6	>2		at least 1	
39782	<1		3–4		>2	<1*		
40098	<1	2–5						>10
40701	<1			3–5	1–2		at least 1	
40899	<1	2–5						
40935	<1	2–5						>10
40941	<1	2–5						
41003	<1		4–5		>2	at least 1		
41065	<1			>6	2			
41082	<1			>6	>2			
41522	<1			3–6	>2			
41582	<1			6	>2			

* This sheep was alive and well at the 30th hour after injection, when it suddenly started to blow, to die in 6 hours. At post-mortem, the lungs were oedematous with a bloody effusion in the pleura-cavity.

These results prove that the injection of formol-toxoid stimulates the formation of antitoxin, resulting in the sheep being able to withstand toxin administered intradermically or intravenously. Further, such sheep resist culture, inoculated intramuscularly. The results of some dozens of tests have shown that the presence in 0·1 c.c. of serum of sufficient antitoxin to neutralize a 1 to 2 M.R.D. of 37 L toxin will render a sheep insusceptible to the administration of 1 to 10 M.L.D. of culture. However, as results will presently be presented showing that sheep may be solidly immunized against culture and yet have no circulating antitoxin (immunization with boiled bacilli), one cannot be certain that their high degree of immunity to culture is necessarily dependent on the antitoxin.

PRODUCTION OF IMMUNITY WITH WASHED BOILED BACILLI.

This experiment was one of the last to be carried out, but is inserted at this point so that the section on the titration of toxoids by the total-antitoxin-combining-power test may be made clearer. Several workers, Robertson and Felix (1930), Green (1929), but in particular Henderson (1932, 1933, 1934), have shown that mice, guinea-pigs and sheep may be immunized against *Cl. chauvoei* bacilli and/or spores by the subcutaneous injection of the killed organisms. Henderson tested the immunity of his immunized mice and guinea-pigs by injecting washed spores activated with calcium chloride. Reasoning on hypothetical grounds, the writer considered that the immunity produced by toxin-(aggressin) free killed germs would be broken down with culture. When activated spores are used for test purposes the antibodies to the bacteria would be sufficient to destroy most of the spores (or the vegetative forms resulting therefrom) but when culture is inoculated, the bacteria could continue to multiply under the activating influence of the toxin, against which no antitoxin is available. As reported by Mason and Scheuber (1936) tests in sheep showed that the injection of killed, washed bacilli produced a powerful immunity against culture, but only when a dense suspension of such bacilli was given. Usually 5·0 c.c. of formolized-whole culture, injected subcutaneously, is sufficient to immunize a sheep against 1 to 20 M.L.D. of culture; the injection of the washed boiled germs of 5·0 c.c. of culture did not immunize against 1 sure M.L.D.

The experiment, the results of which are recorded in Table 9, was conducted along with that noted under "the production of immunity with formolized-filtrate" (Table 8). Strain 64 was grown in the same lot of medium and under the same conditions as noted, and the bacilli from 3 litres were washed 3 times in distilled water, boiled for 2 hours and enough NaCl added to make a 0·85 per cent. concentration. The opacity of the suspension corresponded to tube 9 of a Burroughs, Wellcome and Company's nephelometer. The details of the injections and tests are recorded in Table 9, and should be compared with those given in Table 8.

TABLE 9.

Immunization of Sheep with Washed, Boiled Bacilli. Sheep received 10 c.c. on 12.2.35 and 10 c.c. on 5.3.35 (Experiment 48).

Sheep.	Number M.R.D. toxin neutralized by 0·1 c.c. serum.				Number M.R.D. toxin withstood by sheep.	Number M.L.D. (i.v.) toxin withstood by sheep.		Number M.L.D. (i.m.) of culture withstood by sheep.
	11/2/35.	18/3/35.	27/3/35.	30/3/35.	29/3/35.	28/3/35.	3/4/35.	28/3/35.
34301	<1		<1			<1		
39741	<1	<1						10
39761	<1			<1	<1			
39748	<1	<1						10
40681	<1			<1				
40684	<1				<1		? miss L*	
40711	<1		<1			<1		
40951	<1			<1	<1			
40952	<1			<1	1 not 1½		<1	
41018	<1	<1						
41070	<1			<1	<1			
41110	<1	<1		<1	<1			

It is doubtful if this sheep received a full dose. It survived, after the injection of ? 1 M.L.D.

The results are quite different from those recorded in Table 8. Although the sheep were immune to the intramuscular inoculation of living culture, no anti-toxin was present in the blood and the sheep did not resist the intradermic or intravenous injection of toxin. Thus it is obvious that immunity to culture (and most probably to the natural disease) is not necessarily dependent on the presence of circulating antitoxin. In the two foregoing experiments, only two sheep in each group were tested with culture. However, hundreds of examples could be given of the ability of *one* injection of formol-filtrate to immunize against 1 to 40 M.L.D. of culture and sufficient data were given in the paper by Mason and Scheuber to show that a single stimulus with boiled bacilli produces solid immunity. Further, in these experiments the reaction of all 4 sheep to the administration of 10 M.L.D. was minimal. Normal control sheep were included in each immunity test. The M.L.D. toxin (i.v.) test was not absolutely satisfactory. The lethal dose of the toxin (48) used was 1·5 gm., and from the symptoms shown almost immediately by most sheep, it would appear that the toxin contained a considerable amount of non-specific material. There would appear to be no reason why the further purification of the toxin should not eliminate the appearance of these early symptoms.

In Table 10 are recorded some further tests on the " boiled bacilli " sheep.

TABLE 10.

Development of Antitoxin in, and Resistance to Toxin by Sheep First Treated with Boiled Bacilli (12.2.35 *and* 5.3.35) *and then with Culture* (*i.m.*) *or Toxin* (*i.v.*)

Sheep.	Previous treatment.			30.3.35	No. M.R.D. toxin neutralized by 0·1 c.c. serum.		No. M.R.D. toxin withstood by sheep, 15.4.35
	12.2.35 5.3.35	22.3.35	28.3.35		12.4.35	15.4.35	
39476	Nil	0·5 gm. 48 toxin i.v.				1½	
39741	Boiled bacilli as in Table 9.		10 M.L.D. culture i.m.		·40		
39748			10 M.L.D. culture i.m.		2–3		
39761				1 gm. 48 toxin i.v.		7–10	5
40951				1 gm. 48 toxin i.v.		2–5	5

NOTE.—Sheep 39476 was a " toxin control " sheep.

As would be expected, the intravenous injection of toxin stimulated the formation of antitoxin. It is remarkable that sheep 39741, which received culture, intramuscularly, responded so well, 0·025 c.c. of its serum neutralizing 10 M.R.D. of 37 L toxin (end point not reached).

PRODUCTION OF IMMUNITY AGAINST *Cl. chauvoei* WITH *Cl. septicum* TOXOID.

Under "The specificity of the toxin" it was stated that *Cl. septicum* antitoxin neutralized *Cl. chauvoei* toxin. The relationship of *Cl. septicum* and *Cl. chauvoei* has occasioned discussion and even controversy from the time they were first isolated right up to the present day. Mihailesco (1934) discusses the literature and the *pros and cons* very fully. In the writer's opinion, a considerable amount of the confusion has been caused by workers relying upon morphological and cultural characteristics and failing to conduct a toxin-production test. The supernatant fluid or filtrate of a 24 to 48 hours' meat broth culture of *Cl. septicum* will kill a mouse in from 1 to 24 hours after the intravenous injection of from 0·01 c.c. to 0·1 c.c. No chauvoei filtrate of the writer's has behaved in this way. Further, and of great importance, such septicum toxin is specifically neutralizable by the homologous antitoxin, whereas chauvoei antitoxin has no effect on it. As will be shown shortly, the reverse does not hold good.

Leclainche and Vallée (1900[2]) found that *Cl. chauvoei* antiserum administered to guinea-pigs did not immunize them against *Cl. septicum* culture and those actively immunized against *Cl. chauvoei* were not resistant to *Cl. septicum*. They state that the antiserum (protective and agglutinative) of each germ is rigorously specific. Goss *et al.* (1921) note that chauvoei antiserum protects against *Cl. chauvoei* but not against *Cl. septicum* and that chauvoei agglutinating serum is without effect on septicum bacilli. Basset (1925) noted no cross immunity, and Gins and Hussein (1927, 1928) could not demonstrate cross agglutination. Weinberg and his coworkers (Weinberg and Mihailesco 1929, Weinberg, Davesne, Milhailesco and Sanchez, 1929, Weinberg and Davesne, 1935[1], [2]) maintain that *Cl. chauvoei* and *septicum* are allied micro-organisms. They say that *Cl. chauvoei* produces variants and possesses receptors in common with *Cl. septicum*. Anti-septicum serum nearly always neutralizes *Cl. chauvoei* and although a relatively large volume is necessary, this is usually less than for normal serum. Further, they state, there is a correlation between the amount of chauvoei antitoxin in septicum antiserum and the power of this to neutralize chauvoei culture. Also, they noted cross complement fixation between the two germs.

Experiments with Cl. septicum *antitoxin.*

Using chauvoei toxin 27, it was found that 0·1 to 0·15 c.c. of a certain septicum antitoxin (G.G. 2756, prepared in a horse at the Wellcome Laboratories) neutralized 10 M.R.D. A goat (35451) was immunized at Onderstepoort by injecting septicum anaculture subcutaneously over a period of two and a half months, using the same strain of *Cl. septicum* (K.F.) as was employed to prepare G.G. 2756. Between 0·15 c.c. and 0·2 c.c. of this animal's serum neutralized 10 M.R.D. of chauvoei toxin 27. About 15 mouse (i.v.) M.L.D. of a certain septicum toxin (Batch E) was neutralized by between 0·005 c.c. and 0·01 c.c. of each of these sera. A rather low value septicum antitoxin prepared in a goat by the writer's colleague, Dr. Scheuber, with a local strain (R 27) also neutralized 27 toxin (0·1 c.c. neutralized 3 to 5 M.R.D.). On these results, an experiment was set up to check the matter thoroughly.

Strain K.F. was " single-celled " twice in the manner described by the writer (1936²). The morphological, cultural and pathogenic properties of the resultant culture were those given under " purity of cultures ". It was then grown for 36 hours in meat broth, plus serum, haemolysed cells and liver extract. After removal the meat particles, enough formalin was added to make a 0·45 per cent. concentration, and then incubated for 48 hours at 37° C. The value of the toxin and the toxoid (anaculture) was as follows:—

M.L.D. of toxin (mouse i.v.) = 0·005 c.c. to 0·01 c.c.
M.L.D. of toxoid (mouse i.v.) = >0·5 c.c.
Lv of toxin = 0·1 c.c. bound 0·01 c.c. of antitoxin G.G. 2756.
Total antitoxin-binding value of toxoid = 0·1 c.c. bound 0·0075 c.c. of antitoxin G.G. 2756.

One certain fatal dose of the toxin (mouse i.v.) was not neutralized by 0·1 c.c. of the antitoxins of *Cl. welchii*, *histolyticum*, *oedematiens*, *botulinum* (A, B, C and D), *sordellii* and *chauvoei* (serum of sheep 37281).

Experiment in Sheep(1).—Eight normal sheep were set aside and their sera tested for neutralizing power against the above septicum toxin and against chauvoei toxin (37 L). One M.L.D. of septicum toxin and one M.R.D. of chauvoei toxin was not neutralized by 0·1 c.c. of the serum of any sheep. The animals were then treated as noted in Table II, which also gives the results of various tests.

The spore suspension was prepared from a Dorset's egg medium culture as described by Henderson (1932). The adrenalin was used as an activator of the spores because of the results obtained by the writer (1936¹) with the spores of *Cl. welchii*, Type B. In the second test, the adrenalin was omitted because it was found that toxin, alone, was a suitable activator and did not introduce a non-specific factor.

The results show that two subcutaneous injections of *Cl. septicum* anaculture stimulated the formation of considerable amounts of septicum antitoxin and small amounts of chauvoei antitoxin. After three injections, chauvoei antitoxin was demonstrable in the sera of all five sheep tested. No indication was got of a correlation between the amount of septicum and chauvoei antitoxins in the various sheep. However, there is the definite indication that the amount of circulating chauvoei antitoxin bore a close relation to the amount of toxin (contained in the activated spore suspension) tolerated by the sheep.

TABLE 11.

Production of Immunity against Cl. chauvoei *by Injections of* Cl. septicum *anaculture.*

Sheep.	17/7/34.	7/8/34. 0 1 c.c. serum neutralized		20/8/34. 0·1 c.c. serum neutralized		21/8/34.	3/9/34. 0 1 c.c. serum neutralized		11/9/34. Test with spore suspension i.m.*	12/9/36.	25/9/34. 0·1 c.c. serum neutralized		6/10/34. Test with spore suspension i.m.**
		Septicum. M.L.D.	Chauvoei. M.R.D.	Septicum. M.L.D.	Chauvoei. M.R.D.		Septicum. M.L.D.	Chauvoei. M.R.D.			Septicum. M.L.D.	Chauvoei. M.R.D.	
39412	5 0 c.c. septicum anaculture s.c.	? 1	<1	? 1	<1	5·0 c.c. septicum anaculture s.c.	5–10	? 1		10 c.c. s.c. septicum anaculture	5–10	5–10	L.
40116		? 1	<1	<1	<1		3–4	? 1			>15	? 1	+ 36 h
40390		? 1	<1	<1	<1		1–2	1			2–5	>10	L.
40398		<1	<1	<1	<1		4–5	<1	+ o/n	—	—	—	—
40439		<1	<1	<1	<1		5–10	1½	+ 24 h	—	—	—	—
40463		<1	<1	<1	<1		5 10	1½	+ 36 h	—	—	—	—
40492		1	<1	? 1	<1		5–10	<1		10 c.c. s.c. septicum anaculture	2–5	1–2	L.
40598		<1	<1	<1	<1		4–5	<1			5–10	1½–2	L.

(+ =died ; L=lived. *, ** :—the details of these tests are given in Table 11a. o n=overnight h= hours.)
(The septicum toxin used to detect antitoxin was that noted in the text ; for the detection of chauvoei antitoxin. 37 L was employed.)

Sheep 40116, 0·1 c.c. of the serum of which barely neutralized 1 M.R.D. of 37 L toxin succumbed to the injection of the spore suspension plus 10 mg. of toxin, whereas sheep 40390, 0·1 c.c. of the serum of which neutralized at least 10 M.R.D. of 37 L toxin, withstood spore suspension plus 100 mg. of toxin.

TABLE 11 (*a*).

Details of the Immunity Tests (against Cl. chauvoei) *noted in Table* 11.

Sheep.	Spore suspension.	Adrenalin.	Chauvoei toxin (37 L).	Result.
		11/9/34.		
40398	1·0 c.c.	0·1 c.c.	1·0 mg.	+ o/n.
40439	1·0 c.c.	0·1 c.c.	1·0 mg.	+ 24 h.
40463	1·0 c.c.	0·1 c.c.	1·0 mg.	+ 36 h.
Controls.				
40458	1·0 c.c.	0·1 c.c.	1·0 mg.	+ o/n.
40443	1·0 c.c.	0·1 c.c.	1·0 mg.	+ o/n.
		6/10/34.		
39412	0·5 c.c.		100 mg.	L.
40116	0·5 c.c.		10 mg.	+ 36 h.
40390	0·5 c.c.	not.	100 mg.	L.
40492	0·5 c.c.		50 mg.	L.
40598	0·5 c.c.	used.	50 mg.	L.
Controls.				
34477	0·5 c.c.		5·0 mg.	+ 24 h.
37518	0·5 c.c.		1·0 mg.	+ o/n.
39745	0·5 c.c.		Nil.	L.

Experiment in Sheep (2).—Two sheep received, subcutaneously, in the course of six weeks, 10 injections of a *Cl. septicum* (K.F.) anaculture. Prior to injection, 0·1 c.c. of the serum of neither animal neutralized 1 M.L.D. of a dry septicum toxin or 1 M.R.D. of a dry chauvoei toxin (37 L). At the end of the course of immunization, the following results were obtained:—

	Sheep 37322.	*Sheep* 35540.
Septicum antitoxin ..	0·015 c.c.	and 0·01 c.c. to 0·015 c.c.
	of serum neutralized 20 M.L.D. of a septicum toxin (Batch E).	
Chauvoei antitoxin ...	0·025 c.c. to 0·05 c.c.	and 0·025 c.c. to 0·05 c.c.
	of serum neutralized 5 M.R.D. of 37 L toxin.	
Resistance to i.m. inoculation of chauvoei culture...	5 M.L.D. (0·5 c.c.) lived.	5 M.L.D. (0·5 c.c.) lived.

Controls to culture: Normal Sheep 33849 1 M.L.D. (0·1 c.c.) died 36 hours.
Normal Sheep 37734 2½ M.L.D. (0·25 c.c.) died 24 hours.

(As pointed out by Mason and Scheuber (1936), 0·1 c.c. of an 18 hours' meat-broth culture of *Cl. chauvoei* is usually an M.L.D. for a sheep.)

Experiment in Sheep (3).—Two sheep received 12 subcutaneous injections of septicum anaculture (strain R 27), at bi-weekly intervals. Prior to commencing immunization, no septicum or chauvoei antitoxin was demonstrable in 0·1 c.c. of the serum of either. At the end of the course the following results were obtained:—

	Sheep 37457.	*Sheep* 37913.
Septicum antitoxin ...	0·015 c.c. and	0·015 c.c.
	neutralized 20 M.L.D. of Batch E toxin (made from strain K.F.).	
Chauvoei antitoxin ...	0·07 c.c. to 0·1 c.c. and	0·06 c.c. to 0·07 c.c.
	neutralized 5 M.R.D. of 29 toxin	
Resistance to i.m. inoculation of chauvoei culture.	10 M.L.D. (1·0 c.c.) lived.	10 M.L.D. (1·0 c.c.) died.

Controls to culture: Normal Sheep 38066 1 M.L.D. died.
Immune Sheep 36552 10 M.L.D. lived.

The results of the three experiments just presented prove that in *Cl. septicum* anaculture, there is an antigen capable of stimulating the formation in sheep of *Cl. chauvoei* antitoxin and of rendering these animals insusceptible to the inoculation of several lethal doses of Cl. chauvoei culture (or activated spore suspension).

Having shown that *Cl. septicum* antigens immunized against *Cl. chauvoei*, it became necessary to prove or disprove that the reverse held good.

Experiment 1.

Eight sheep which had received subcutaneously from 2 to 20 c.c. of *Cl. chauvoei* anaculture and had survived the intramuscular inoculation of one or more M.L.D. of culture were bled and their sera titrated for the presence of septicum antitoxin. One sure fatal dose (0·01 c.c.) of a certain septicum toxin (Batch E) was not neutralized by 0·1 c.c. of any serum, and a doubtful lethal amount, 0·007 c.c., was neutralized by only 2 sera. In a more detailed test, it was found that the sera of four of these sheep had no more neutralizing effect upon fractional M.L.D. of septicum toxin than had the sera of 4 normal sheep.

Experiment 2.

Three sheep were treated as follows. One (37534) received chauvoei anaculture and then living culture (1 to 2 M.L.D.) and two others (37827, 36552) filtrated black quarter oedema fluid (from sheep) and then living culture (10 M.L.D.). Between 5.4.34 and 21.6.34 each received 9 gm. of a dry chauvoei toxin and 1,550 c.c. of a formol-filtrate subcutaneously. On the 4.7.34, 0·1 c.c. of the serum of no sheep neutralized 1 M.L.D. of septicum (Batch E) toxin. Of the pooled sera of the three animals 0·005 c.c. neutralized 10 M.R.D. of chauvoei 37 L toxin. On the 10.7.34 each sheep received a lethal dose of septicum toxin intravenously, and all died within 12 hours. The amount given was 3·5 mg. of dry toxin per kilogram body weight. Using the same batch (E) of toxin, Mason (1935) found that the lethal dose was about 3 mg. per kilogram body weight.

These two experiments and especially the second one show that sheep highly immune to *Cl. chauvoei* have no more immunity than normal sheep to the toxin of *Cl. septicum*.

PART III.

THE TITRATION OF CL. CHAUVOEI ANTIGENS.

It is accepted in immunology that, with a few exceptions, the more antitoxin a toxin or toxoid binds, the greater is its antigenic power. With diphtheria toxoid, the flocculation titre is closely related to immunizing power and the writer (1936[1]) showed that the total-antitoxin-binding-power of *Cl. welchii*, Type B toxoid bears a close relation to its power of immunizing guinea-pigs. Thus, there was considerable *a priori* grounds for assuming that the same would hold good for *Cl. chauvoei* formol-toxoid or anaculture.

Scott (1923[1]) evaluated filtrates by their power of activating non-lethal doses of *Cl. chauvoei* spores or bacilli. Another method he employed (1923[2]) was to estimate the value of the filtrate in terms of antitoxin. Filtrate and serum were mixed, and living spores added, and the whole inoculated into guinea-pigs. A "good" filtrate would bind more antitoxin than a "bad" one and thus less antitoxin would be left over to "neutralize" the spores and the animals would die. Apart from the fact of whether or not this is a suitable method of titrating filtrates for antigenicity (and the results to be presented do not support the contention), the writer (unpublished work) was unable to obtain consistent results with Scott's method; the guinea-pigs died in a most irregular fashion, irrespective of the amounts of antiserum or filtrate given. In a later article, Scott (1930) states that guinea-pigs are unsuitable test animals since they vary in susceptibility to *Cl. chauvoei*. He also says that the addition of formalin to filtrate increases its potency, but Zschokke (1932) shows that this is due to the irritating effect of the formalin, itself.

At these laboratories, the method of testing the immunizing value of black quarter vaccines is to inject 2 c.c. to 20 c.c. into sheep and to test with living culture after 14 to 21 days. This method gives a result, sufficient for practical purposes—a vaccine which so immunizes a group of six sheep that they withstand 1 to 5 lethal doses of culture is suitable for field use. However, only the roughest of comparisons between two vaccines is possible, unless an unwieldly number of animals is used for the test. One does not know the value of the antigen prior to injection so that it is mere chance if the correct amount of culture to show up a difference is inoculated at the test. This point was brought home forcibly to the writer and his colleague, Dr. Scheuber, in a series of experiments with black quarter vaccines.

About all that the method does tell is whether a vaccine is good or bad, i.e. it is good if 5 or 6 of 6 injected sheep withstand 1 to 10 M.L.D. of culture and bad if 4 to 6 of 6 sheep die after the inoculation of 1 or 2 M.L.D.

To use guinea-pigs instead of sheep would not help, if the method of test was to be the inoculation of living culture. However, on the assumption that immunity production was bound up with the amount of antitoxin an antigen could stimulate into formation, the guinea-pig promised to be a handier, but no better, animal than the sheep.

Experiment 1.

An experiment was carried out in the following way:—One group of guinea-pigs received, as their primary stimulus, the injection of a dry toxin, and as their secondary stimulus, a good or a bad vaccine. A second group received, as their primary and their secondary stimulus, the good or the bad vaccines and a third lot, the dialysed ammonium sulphate precipitates of these vaccines. (For the meaning of "good" and "bad", see above.) The results of the tests are given below.

Group 1:

Scheme. { Primary stimulus—30 mgm. of chauvoei toxin (S.C.) subcutaneously.
Secondary stimulus—2·5 c.c. of good or bad vaccine subcutaneously.

5.1.34: S.C. toxin injected. All animals developed a swelling with reddening and 3 died.

25.1.34: 6 guinea-pigs bled and the sera pooled; 0·1 c.c. of serum did not neutralize 1 M.R.D. of toxin 29. Two-guinea pigs each received 1 M.RD. of toxin 29; both reacted.

26.1.34: Half of the group received the good vaccine and the other half the bad vaccine.

5.2.34: The guinea-pigs of each sub-group were bled and sera pooled; 0·1 c.c. of serum was titrated against toxin 29. From 3 to 6 M.R.D. were neutralized by the serum of the "good vaccine" group and 2 to 3 M.R.D. by the "bad vaccine" group. Each guinea-pig now received intradermic injections of toxin 29, 1, 1½, 2, 2½, 3½ and 5 M.R.D. There was no significant difference as groups: most animals resisted 2 M.R.D. and some 5 M.R.D. If a difference was to be read into the reactions, it was in favour of the "bad vaccine" group.

Group 2:

Scheme. Primary and secondary stimulus—good or bad vaccine.

5.1.34: Guinea-pigs received subcutaneously 2·5 c.c. of vaccine.

25.1.34: Animals bled as sub-groups and sera pooled; 0·1 c.c. of the "good" sera almost neutralized 1 M.R.D. of 29 toxin and 0·1 c.c. of the "bad" sera gave a doubtful reaction. Two guinea-pigs from each sub-group which received 1 M.R.D. of 29 toxin reacted.

26.1.34: Vaccine re-injected into the guinea-pigs (2·5 c.c.).

5.2.34: Animals bled as sub-groups and 0·1 c.c. of serum tested against 29 toxin; between 2 and 3 M.R.D. were neutralized by the " bad group " serum and 3 to 4 M.R.D. by the " good group " serum. The four guinea-pigs of each sub-group now received 1, 1½ and 2 M.R.D. of 29 toxin intradermically. Three of the " bad " lot reacted to 1 M.R.D. and one doubtfully; two of the " good " lot reached to 1 M.R.D., one resisted 1 M.R.D. and reacted doubtfully to 2 M.R.D. and the fourth guinea-pig reacted doubtfully to 1 and 2 M.R.D

Group 3.

Scheme { Primary and secondary stimulus—the dried dialysed ammonium sulphate precipitates of the bad and the good vaccines. The same volumes of anacultures were precipitated. The yield of powder from the good vaccine was 6 gm. and of the bad vaccine 0·6 gm.

9.1.34: Guinea-pigs received, subcutaneously, 40 mg. of dry vaccine.

25.1.34: Animals bled as sub-groups and sera pooled; 0·1 c.c. of the " bad " sera partially neutralized 1 and 1½ M.R.D. of 29 toxin and 0·1 c.c. of the " good " sera did not neutralize 1 M.R.D. Two guinea-pigs from each group were tested intradermically, with 1 M.R.D. of 29 toxin—the two bad vaccine animals partially resisted this amount and the good vaccine guinea-pigs did not.

26.1.34: Re-injections of vaccine (40 mg.) carried out.

5.2.34: Guinea-pig bled as sub-groups and sera pooled; 0·1 c.c. titrated against 29 toxin. The " bad " sera neutralized 5 M.R.D. and the " good " sera 1 M.R.D. On testing each animal with toxin intradermically, one of the " bad " group resisted 2 M.R.D., another doubtfully 2 M.R.D. and the third doubtfully 1 M.R.D.; not one of the four animals of the " good " group resisted 1 M.R.D.

Comment.—The results of this experiment are unsatisfactory. Apparently, the primary stimulus of toxin (Group 1) produced such a high basal immunity that the relatively weak antigenic vaccines did not stimulate the production of antitoxin to an extent sufficient for differences to be shown up. Two injections of the anacultures (Group 2) showed up no significant differences in antitoxin production. A significant difference was got when the dialysed ammonium sulphate precipitates of the good and the bad vaccines were used, the bad vaccines stimulating the production of more antitoxin than the good one. The reason for this lies probably in the yields of dry powder obtained. Although equal volumes were precipitated, the weight of the " bad " dry vaccine was ten times less than that of the good. Thus, the probability exists that, as equal amounts of each dry vaccine were injected, 10 times more " bad " than " good " antigen was administered. However, no indication was got that an anaculture which immunized sheep well against the inoculation of culture stimulated the production in guinea-pigs of more antitoxin than did an anaculture which immunized sheep badly.

Experiment 2.

Four vaccines were chosen on the basis of their having produced bad, fair, fairly good and good immunity (to culture) in sheep. Into 4 groups of guinea-pigs, these vaccines were injected (2·5 c.c. subcutaneously, twice, at 21 days' interval). Ten days after the second injection, the animals were bled as groups and each individual tested intradermically with toxin. Significant differences were not got. Further, 4 groups of 3 sheep each (12 sheep in all) received subcutaneously 5·0 c.c. of one or the other vaccine (twice at 16 days' interval). Ten days after the second injection the sheep were then bled as groups, their sera pooled, and titrated against toxin 27, intradermically in guinea-pigs. The results of these titrations and of the final immunity test with culture are given in Table 12.

TABLE 12

The Amount of Antitoxin Produced by Sheep after Two Subcutaneous Injections of Bad, Fair, Fairly Good and Good Vaccines. Also the resistance of these Sheep to the i.m. Inoculation of Culture.

Sheep.	13/12/34.	29/12/34.	8/1/35. Group serum pooled 0·1 c.c. neutralized 27 toxin M.R.D.	16/1/35. Resistance to culture i.m.	
				Dose c.c.	Result.
Bad vaccine—	5·0c. c. vaccine s.c.	5·0 c.c. vaccine s.c.			
37000				2·0	+ 24 hr.
37703			1–1½	2·0	+ 5 d.
37731				2·0	+ o/n
Fair vaccine—					
37877				6·0	Lameoedema.L
38183			4	6·0	+ o/n.
38188				4·0	+ 3 d.
Fairly good vaccine—					
38189				2·0	L.
38196			<2	2·0	L.
38198				4·0	L.
Good vaccine—					
38212				4·0	L.
38214			10	6·0	L.
38216				4·0	L.
Controls to culture injection—					
34732				0·2	+ o/n.
34721				0·1	+ 20 h.

(+ =died, L=lived, hr.=hours, d=days, o/n=overnight.)

If only the good and the bad vaccines had been used, the result of the test would have been considered highly satisfactory; 0·1 c.c. of the " bad " serum neutralized only 1 to 1½ M.R.D. of toxin and not one of the 3 sheep was immune to 20 M.L.D. of culture whereas 0·1 c.c. of " good " serum neutralized 10 M.R.D. of toxin and the sheep resisted 40 to 60 M.L.D. of culture. However, the introduction of the fair and fairly good vaccines shows that a correlation between the amount of circulating antitoxin and the amount of culture resisted does not exist. The pooled serum (0·1 c.c.) of the sheep immunized with the fairly good vaccine did not neutralize 2 M.R.D. of toxin (and, from the intensity of the reaction, probably would not neutralize 1 M.R.D.), yet the sheep withstood 20 to 40 M.L.D. of culture. It should be stated that the values (" good ", " bad ", etc.) placed upon the vaccines were arrived at from the results of a preliminary culture immunity test on sheep. Further, the reason why the same amount of culture (see Table 12) was not inoculated into each sheep was because it was anticipated that the antitoxin titration would run parallel to the culture-resistance test.

Experiment 3

If immunity to *Cl. chauvoei* culture depended on the presence of circulating antitoxin, then one could, with justification, assume that the amount of antitoxin a formol-toxoid (anaculture) bound would bear a relation to the immunity produced by it. Attempts to ascertain the total-antitoxin-combining-power (T.C.P.) of liquid toxoids were not altogether satisfactory, because, in many cases, too little antitoxin, to allow of accurate results, was bound. However, by using dialysed ammonium sulphate precipitates, values could be placed on the toxoids. The technique was as follows. The Lr of a dry toxin was accurately established, and this toxin and the antitoxin were used throughout the tests. The dry toxoid, dissolved in saline, was left in contact for one hour at room temperature with a certain amount of antitoxin. Thereupon, to a series of tubes containing this mixture, fractions of an Lr dose of toxin were added and after a further half-hour at room temperature, the toxoid-antitoxin-toxin mixtures were injected, intradermically, into guinea-pigs. In a typical titration, those mixtures containing small amounts of toxin produced no reactions, whereas those with larger amounts did so. Knowing the Lr of the toxin, a simple calculation showed how much antitoxin the toxoid had bound. Further, knowing the volume of toxoid originally precipitated and the weight of the dry powder, one could calculate the amount of antitoxin that 1·0 c.c. of liquid toxoid would bind. In the first half of this experiment one lot of sheep received, subcutaneously, 5·0 c.c. of anaculture, 86 C, and the other lot 5·0 c.c. of anaculture 87. The details of the test are given in Table 13.

Toxoid 86 C bound more antitoxin than toxoid 87 and stimulated the formation of more antitoxin *in vivo*. However, the immunity test with spores allows of no conclusion being drawn. All the sheep survived the test.

TABLE 13

Experiment to Ascertain if there is a Correlation between the T.C.P. of a Toxoid, the Tolerance to Toxin and to Culture (Activated Spores) of Sheep Immunized with it and the Amount of Circulating Antitoxin in the Sheep.

Toxoid.	Sheep.	Value of toxoid.	Tolerance to toxin (37 L) i.d. M.R.D.	No. M.R.D. of 37 L toxin neut. by 0 1 c.c. serum.	Resistance to toxin-activated spore suspension.	
					Amount toxin (37 L).	Result.
86 C	39309 39684 39964 40543	1 0 c.c. liquid toxoid bound 0 0008 c.c. A.T. 38212	Not done.	1 5 5 2	50 mg. 100 mg. Not done. 100 mg.	L. L. L.
87	40402 40527 40779 41004	1 0 c.c. liquid toxoid bound 0 0004 c.c. A.T 38212	<1 ? 1 1 <1	<1 1 ? 1 <1	50 mg. 100 mg. 100 mg. 50 mg.	L. L. L. L.
Controls	34477 2771[illegible]		<1 <1	<1 <1	1 mg. 5 mg.	+ o/n. + 24 h.

(L=lived, + =died, o/n=overnight. h=hours, neut.=neutralized. T.C.P.=total antitoxin-combining-power.)

The toxoid was injected subcutaneously, and the toxin tolerance and the serum neutralization tests carried out 3 weeks later. The resistance to activated spore test was carried out after a further 10 days.

Experiment 4.

The details of this test are given in Table 14. It was carried out as noted under Experiment 13, with the difference that culture, instead of activated spores, was used to test immunity.

TABLE 14.

Experiment to Ascertain if there is a Correlation between the T.C.P. of a Toxoid, the Tolerance to Culture of Sheep Immunized with it and the Amount of Circulating Antitoxin in the Sheep.

Toxoid.	Sheep.	Value of toxoid.	No. M.R.D. 37 L toxin neutralized by 0·1 c.c. serum.	Resistance to culture c.c.
88	36881	1·0 c.c. of liquid toxoid bound 0·0005 c.c. of A.T. 38212	? 1	0·5 L.
	38934		1	1·0 L.
	38937		? 1	0·5 L.
	38940		1½	1·0 L.
89A	38881	As 88.	<1	0·5 L.
	40241		>1½	1·0 L.
	41000		>1½	0·5 L.
	41092		>1½	1·0 L.
91	41008	1·0 c.c. of liquid toxoid bound 0·00016 c.c. A.T. 38212.	<1	1·0 L.
	41028		<1	0·5 L.
	41112		<1	2·0 L.
	41131		<1	2·0 L.
Controls	41022	•	<1	0·1 + o/n.
	41047		<1	0·25 + 24 hr.

(See notes under Table 13.)

The results do not indicate that there is a correlation between the amount of circulating antitoxin, the T.C.P. of the toxoid and the amount of culture borne by the sheep. Toxoid 91 bound about one-third of that of toxoid 89A, circulating antitoxin was not demonstrated in the immunized sheep, yet these sheep resisted 5 to 20 M.L.D. of culture. It is realized that all the sheep resisted the culture inoculation and thus a true comparison cannot be made. However, some years' experience with toxoids as noted in Table 14 has shown that it is unusual to find them immunizing against more than 20 M.L.D., the usual amount being between 2 and 10 M.L.D.

EXPERIMENT 5.

To be certain of having toxoids of widely differing values (T.C.P.), a liquid formol-filtrate was used in the following manner. Sheep received, subcutaneously, 5·0 c.c. of the toxoid, unheated, heated (60° C. half-an-hour), boiled (95° C. half-an-hour) and autoclaved (120° C., 15 pounds pressure, half-an-hour). The T.C.P. was worked out in the manner described. Table 15 records the experiment.

TABLE 15.

The Correlation between the T.C.P. of Toxoids and the Production of Antitoxin by, and the Resistance to Culture of Sheep Immunized with them.

Toxoid.	Sheep.	Value of toxoid.	No. M.R.D. 37 L toxin neutralized by 0·1 c.c. serum.	Resistance to culture c.c.
Not heated	39354 41099 40949 41058	1·0 c.c. bound 0·0005 c.c. A.T. 38212	<1 <1 ? 1 <1	0·5 oed. L. 1·0 oed. L. 2·0 + 5 d. 4·0 + 2 d.
Heated 60° C, ½ hour	40945 40993 40729 41050	1·0 c.c. bound 0·0001 c.c. A.T. 38212.	? 1 <1 <1 <1	0·5 L. 1·0 L. 2·0 + 5 d. 4·0 L.*
Heated 95° C, ½ hour	41087 41097 40959 40976	1·0 c.c. bound <0·0001 c.c. A.T. 38212	<1 <1 <1 <1	0·5 + 2 d. 1·0 L.* 1·0 + 1 d. 2·0 + o/n.
Autoclaved, ½ hour	41063 41020 39652 40514	As 95° C. toxoid.	<1 <1 <1 <1	0·5 + 2 d. 1·0 + 2 d. 1·0 + o/n. 2·0 + o/n.
Controls	41125 41103		<1 <1	0·1 + 1 d. 0·25 + 1 d.

(L = lived, + = died, d = days, oed. = oedema.)

* Sheep 41050 and 41097 had oedema of the leg (the site of inoculation) down to the fetlock, the skin was blue in places and a haemorrhagic fluid exuded from the skin. Careful nursing prevented their death.

The sheep received 5·0 c.c. of toxoid subcutaneously, the serum neutralization test was done 21 days after and the culture test 2 days after this.

The results given in Table 15 show that the antitoxin-binding power of a toxoid has no bearing on its ability to immunize sheep against the inoculation of culture. The heating of the toxoid at 60° C. reduced its binding power 5 times, yet its immunizing power (to culture) was not altered. Further, it is of definite significance that sheep 41087, 41063 and 41020 died after 48 hours and that sheep 41097 survived. A dose of 0·1 c.c. of culture killed a control animal in 24 hours; 5 to 10 times this amount would kill, with certainty, overnight. Unfortunately, the antitoxin production, even by the unheated toxoid, was so feeble that a comparison cannot be made. The experiment indicates that, in *Cl. chauvoei* formol-filtrates, there is an antigen much more stable than toxin capable of immunizing against culture. (It will be recalled that toxin is destroyed after half-an-hour's heating at 60° C.).

Experiment 6.

In this experiment, the L strain of *Cl. chauvoei* was used. As previously stated, this culture, as originally received, was nearly non-pathogenic for guinea-pigs but by passing it through guinea-pigs (culture activated with adrenalin) a virulent strain was obtained. Toxin was made from a 36 hours' culture of the avirulent and of the virulent strain. Toxoids were made from each by adding 0·4 per cent. formol and incubating for 48 hours. The T.C.P. of the toxoids were established, sheep received 5·0 c.c. subcutaneously, were bled 21 days later and 2 days later still were tested with culture. Table 16 records the results.

TABLE 16.

The Correlation between M.L.D. and Lr of the Original Toxin, the T.C.P. of the Formol-toxoid Produced from it, the Amount of Antitoxin formed in Sheep into which the Toxoid is Injected and the amount of Culture Resisted by the Sheep.

Toxoid.	M.R.D. toxin.	**Lr toxin.	Value of toxoid.	Sheep.	No. M.R.D. 37 L toxin neut. by 0·1 c.c. serum.	Resistance to culture M.L.D.
From avirulent culture	? 0·1 c.c.	<0·0003 c.c.	1·0 c.c. bound 0·0001 c.c., A.T. 38212	41510	<1	5 + 2 d.
				41054	<1	10 + 2 d
				41513	<1	20 L.
				41521	<1	20 L.
From virulent culture	at least 0·01 c.c.	>0·003 c.c.	1·0 c.c. bound 0·0045 c.c., A.T. 38212	41527	<1	10 + 2 d.
				41534	? 1	20 + 1 d.
				41500	>1½	30 + 1 d.
				41088	>1½	40 L.*

(+ = died, L = lived, d = days.)

* The leg of this sheep was oedematous, the skin blue and oozing a haemorrhagic fluid.

** Liquid toxin used ; test dose=0·2 c.c. ; the figure given is the amount of antitoxin 38212 required to neutralize this amount.

The results show that there is no correlation between the antitoxin-binding value of a toxoid and the resistance to culture produced by it in sheep. The toxoid from the virulent culture bound 45 times more antitoxin than that from the avirulent, 3 of 4 sheep had demonstrable circulating antitoxin, yet (although the doses of culture inoculated are not exactly the same) the sheep were no more immune to culture than those into which the " avirulent " toxoid was injected.

Experiment 7

In Table 13 (experiment 3) it is shown that toxoid 86 C, 1·0 c.c. of which bound 0·0008 c.c. of antitoxin 38212, stimulated the formation of more antitoxin, in sheep, than toxoid 87, 1·0 c.c. of which bound 0·0004 c.c. of the same antitoxin. Also in experiment 6, it is shown that the toxoid of the virulent strain bound 45 times more antitoxin than that of the avirulent strain and stimulated the formation, in sheep, of more antitoxin. In the experiment now to be noted, 3 precipitated toxoids were used. Their T.C.P. were established, guinea-pigs received 2 injections (200 mg. each time) subcutaneously (23 days' interval), their pooled sera (as groups) titrated for antitoxin 14 days after the second injection and three days after this, all guinea-pigs received a lethal dose of toxin intravenously. The object was to ascertain if there was a correlation between the binding-value of a toxoid, the amount of antitoxin produced, and toxin tolerance. Table 17 records the results.

TABLE 17.

The Correlation between the T.C.P. of a Toxoid, Antitoxin Formation and Toxin Tolerance.

Toxoid.	* Value.	No. M.L.D. 37 L toxin neut by 0·1 c.c. pooled serum.	Toxin (37 L) tolerance 1 M.L.D.
90 autoclaved	<0·0001 c.c.	<1	4 † 1 L.
91	0·0001 c.c.	<1	3 † 1 L.
46	0·0035 c.c.	5	0 † 5 L.

(† = died, L = lived.)

* Value = 20 mg. toxoid bound the amount of antitoxin 38212 noted.

This experiment proves that the total-antitoxin-combining-value of a toxoid is closely bound with the amount of antitoxin stimulated into formation and with the toxin tolerance of animals immunized.

Experiment 8.

This experiment concerns the production of immunity in sheep with oedema fluid, the so-called natural aggressin. It is well known that the oedematous fluid produced by inoculating *Cl. chauvoei* into cattle, is a powerful immunizing agent.

In this experiment, oedema fluid was collected from a number of sheep, just dead from experimental black quarter. After filtration though a Berkefeld N candle, an attempt was made to carry out a binding-power test. No antitoxin was bound. The dialysed ammonium sulphate precipitate bound no antitoxin. No antitoxic property was detected in the fluid. However, as was to be expected, both the oedema fluid and its precipitate immunized sheep—of 8 sheep which received the one or the other material, two withstood 5 M.L.D. of culture, three 10 M.L.D., one 20 M.L.D., one died from 20 M.L.D. and one from 10 M.L.D.

Experiment 9.

At the time this experiment was started, it was known that boiled bacilli produced a solid immunity, that heated toxoid produced a certain degree of immunity and that the binding power of a toxoid and the amount of circulating antitoxin in an animal had no connection with the amount of culture that would be borne. It was difficult to apportion to the toxin (or its atoxic modification) and the heat stable antigen in a toxoid their respective antigenic values but the indications were that the heat stable fraction was the more important. Therefore, an attempt was made to titrate the stable antigen. The bacilli from 25 litres of a 24 hours' culture were obtained. The culture was passed through a pulp, the pulp soaked in a small volume of saline and the bacilli squeezed out in a press. The bacilli were then washed by spinning three times (with changes of saline solution) at 4,000 revolutions per minute. The dense washed suspension, 200 c.c. in amount, was frozen (- 15° C.) and thawed (20°–26° C.) 5 times in 5 days, and spun until clear. A slightly opalescent fluid was obtained. No reaction was produced when 0·1 c.c., in 0·3 c.c. of 1/100 adrenalin, was injected, intradermically, into guinea-pigs nor did 0·5 c.c. kill a mouse on intravenous injection. The fluid was fanned down to 80 c.c. Of this 0·2 c.c. produced no reaction in a guinea-pig, nor did 0·5 c.c. kill a mouse. Reduction with sodium hydrosulphite did not produce a reacting or toxic product. No antitoxin-binding-power could be demonstrated. Using an antitoxic serum (38212) no specific precipitation could be shown up. This antitoxin probably contained plenty of the antibody to the heat stable antigen. No precipitation was got on mixing the fluid with sera produced in sheep by injections of boiled bacilli.

An immunity test was carried out in sheep. One lot of sheep received the supernatant fluid, and the other lot the supernatant which had been boiled for 2 hours.

No precedent existed for the amount of culture that a sheep immunized with supernatant fluid would withstand. Thus, it is possible that sheep 41523 and 41555 would have survived more than 2½ and 1 M.L.D. respectively. Further, since one injection produced a solid immunity, the testing of the immunity after 2 injections was not carried out. One injection of the boiled supernatant did not so immunize 2 sheep that they withstood 2½ and 1 M.L.D. of culture respectively. However, 2 injections produced a solid immunity. One injection of neither antigen stimulated the formation of antitoxin; two stimuli with the unboiled supernatant led to the formation of a considerable amount of antitoxin but with the boiled material, two injections did not have this effect.

Thus, from this experiment one may infer the presence, in the frozen and thawed supernatant fluid of a washed *Cl. chauvoei* suspension, of two antigens, viz., one which is heat labile and capable of stimulating the formation of antitoxin and the other, heat stable, incapable of inducing the formation of antitoxin but capable of immunizing sheep against culture. The possibility exists that the heating of the antigen destroyed some of the heat stable material.

TABLE 18.

The Immunizing Power of the Unboiled and the Boiled Supernatant of Frozen and Thawed Cl. chauvoei *Bacilli.*

Sheep.	Material.	22/2/35.	13/3/35. No. M.L.D. 37 L toxin neut. by 0·1 c.c. serum.	13/3/35. No. M.L.D. of culture resisted.	14/3/35.	4/4/35. No. M.R.D. 37 L toxin neut. by 0·1 c.c. pooled serum.	5/4/35. No. M.L.D. of culture resisted.
41523	Supernatant.	5·0 c.c. s.c.	<1	2½			
41538			<1	N.T.	5·0 c.c. s.c.	5–10	N.T.
41555			<1	1	5·0 c.c. s.c.	5–10	N.T.
41563			<1	N.T.			
37385	Boiled Supernatant	5·0 c.c. s.c.	<1	<1			
37397			<1	N.T.	5·0 c.c. s.c.	<1	5
38891			<1	N.T.	5.0 c.c. s.c.	<1	2½
41532			<1	<2½			

(s.c.=subcutaneously, N.T.=not tested.)

At the time of both culture immunity tests control sheep were inoculated. The M.L.D. is based on the smallest amount required to kill them.

It is to be noted that although unheated supernatant stimulated antitoxin formation, no toxin or toxoid was demonstrable in it by the T.C.P. titration. An absolutely satisfactory explanation for this cannot be given. It may be that the amount of antigen was too small to allow of its being detected by the method of test. Some little support for this is offered by the fact that after one injection, not a trace of antitoxin was detectable in any one of the four sheep.

DISCUSSION.

The results presented in Part I, prove that, in the filtrates of 18 to 24 hours' cultures of *Cl. chauvoei*, a toxin is present. This toxin has been demonstrated on previous occasions, in particular by Kojima and by Kerrin, but their method of titrating it, viz. the intravenous injection of mice, was, in the writer's opinion, unsatisfactory. When from 0·1 c.c. to 0·5 c.c. is necessary to cause death in mice and when death may occur in a few seconds or minutes, great difficulty is experienced in conducting neutralization tests. The intradermic titration method in guinea-pigs, using dried dialysed ammonium sulphate precipitates, allowed of the obtaining of a satisfactory minimum reacting dose and further, a conveniently sized test dose, containing 5 to 10 reacting doses, could be used. However, satisfaction was not obtained until adrenalin was added to the toxin or the toxin-antitoxin mixtures. The effect of this substance appeared to be the localization of the toxin in the tissues for a time sufficiently long for it to produce visible damage. Without it, the toxin caused an early-appearing intense reddening of the dermis, this disappearing to a greater or less extent in a few hours, so that after 18 or 24 hours, only a red flush marked the site of injection. With adrenalin, the reaction was clearly readable as an intense red mark. That this toxin was the product of *Cl. chauvoei* was shown by its being neutralizable by homologous antitoxin. The specificity of the toxin is not detracted from because the sera of some normal rabbits and bovines were able to neutralize a few M.R.D. Weinberg and his colleagues have already noted this fact. It is possible and even probable that in the case of bovines their antitoxin is the result of stimuli with *Cl. chauvoei* but with rabbits this explanation can hardly be invoked since guinea-pigs, living under the same conditions do not develop antitoxin. However, it is difficult to understand why bovines receive stimuli and sheep do not. It may be that it is an inherent characteristic of the animal to produce antitoxin and not the effect of specific stimuli but this, it is agreed, is merely a hypothetical statement and not an explanation.

Weinberg has insisted for many years that *Cl. septicum* antitoxin may neutralize *Cl. chauvoei* culture. The results presented confirm this, in as much as septicum antitoxin neutralizes chauvoei toxin and sheep may be solidly immunized against *Cl. chauvoei* culture by the use of septicum antigens.

Whilst a haemolysin was demonstrable in filtrates, it was usually of low titre, whereas other workers, for example Kerrin, obtained much higher values. Possibly this is to be explained by the use of different media. However, the writer could not show that the haemolysin was the same as the toxin—more antiserum was required to neutralize the same volume of lysin as toxin. However, it is admitted that the same attention was not given to the study of the lysin as of the toxin.

The study on the production of immunity was disappointing because a method of titrating antigens apart from their immunizing power was not obtained. The writer had the preconceived idea that the toxin (or toxoid) of *Cl. chauvoei* would be, in great measure, responsible for the production of immunity. If such were the case the filtrate or toxoid binding most antitoxin would produce the strongest immunity. Experiments showed that the total-antitoxin-binding-value of a toxoid had a direct bearing on the amount of antitoxin produced in an animal into which it was injected but was not correlated with the resistance produced to living culture. Boiled, washed *Cl. chauvoei* which bound no antitoxin produced a high degree of immunity in sheep when they were tested with culture, but no antitoxin was demonstrable nor were the sheep resistant to toxin injected intradermically or intravenously. Thus one could conclude that two antigens were involved in the production of immunity, one a heat labile toxin and the other a heat stable antigen. Basset (1925^2) pointed out that the heating of formol-toxoids at 60° C. for one hour did not destroy their antigenic value and Viljoen and Scheuber (1926) found that artificial aggressin (filtrate) does not lose all of its immunizing value even when exposed to 95° C. for half-an-hour. Ramon (1928), Van Gerderen (1933) and Povitzky (1935) show that tetanus and diphtheria toxoids may be heated at 60° C. to 70° C. without demonstrable loss in immunizing value. This has been borne out in the present investigation with *Cl. chauvoei* formol-toxoids. Heating at 60° C. for half-an-hour had no detrimental effect on immunity production and even boiled toxoid had a slight immunizing value. As toxin and toxoid after half-an-hour at 60° C. bind no or only a trace of antitoxin, it would appear that the heat stable antigen is of great importance in the production of immunity to *Cl. chauvoei*. Lourens (1935) expressed the interesting view that, as the old spore vaccines for black quarter were made from heated infected muscle, the immunity produced was due to the aggressins in the dry powder. The present work would tend to confirm this view. The relatively small number of killed germs in the dry muscle powder would hardly be sufficient to set up immunity and it is a very moot point if the immunity was due to the *in vivo* germination of an odd, so-called attenuated spore.

Finally, the virulence of a *Cl. chauvoei* culture need not necessarily have a bearing on the antigenicity of a formol-filtrate prepared from it. The immunity produced by the toxoids of a virulent and of a relatively avirulent culture of the same strain was of about the same order. This is supported by Haslam and Lamb (1919) and Gräub and Zschokke (1910) who stated that the immunizing power of filtrates was independent of their toxicities.

It would appear that no finality can be reached on the production of immunity to *Cl. chauvoei* until a method is devised for titrating the heat stable antigen and the antibody produced by it. Since (1) antigens, such as boiled bacilli and heated toxoids free from demonstrable toxin or toxoid can produce a high degree of immunity to culture, (2) since there is no correlation between the antitoxin-binding power of an antigen and its ability to produce resistance to culture and (3) since the absence of circulating antitoxin is no indication of the inability of an animal to resist culture, it is probable that the heat stable antigen is the important one.

Until toxin (aggressin) or toxoid free from the heat stable antigen is obtained, one cannot say whether it plays any part in the production of immunity to the inoculation of living culture or to the natural disease.

Although the writer has gained the impression, from a study of the literature, that many workers suspected the presence of a heat stable antigen, credit must go to Henderson for having so clearly shown that the antigen in boiled bacilli is such a powerful immunizing agent.

CONCLUSIONS.

(1) In culture filtrates of *Cl. chauvoei* two antigens have been demonstrated (*a*) a toxin, and (*b*) a heat stable antigen. The toxin can best be demonstrated by injecting filtrate intradermically into guinea-pigs, particularly if adrenalin is incorporated in the filtrate. The presence of the heat stable antigen is proved if toxin-free (heated) bacilli are injected into sheep; after a suitable interval these are immune to the inoculation of living culture.

(2) The toxin, or its atoxic modification, stimulates the formation of antitoxin when injected into animals, whereas the heat stable antigen does not do so.

(3) The toxin is neutralizable by homologous antitoxin, by *Cl. septicum* antitoxin and to a slight extent by the sera of some normal bovines and rabbits.

(4) The toxins of four different strains of *Cl. chauvoei* have been found to be indistinguishable serologically.

(5) There is no correlation between the amount of circulating antitoxin in an animal and the power of the animal to resist the inoculation of living culture.

(6) There is a correlation between the amount of circulating antitoxin in an animal and its power of resisting toxin, injected intravenously or intradermically.

(7) The evidence points to the heat stable antigen being the important one in the production of immunity to *Cl chauvoei* culture.

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Intradermic Reaction in Guinea Pig
2 M.R.D. Toxin, 24 hours.

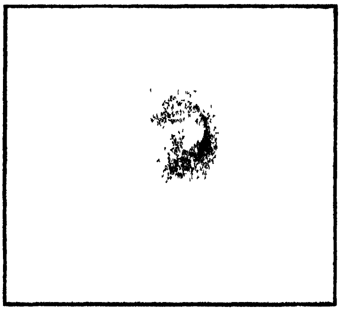

Fig. 1

Section III.

Plant Studies AND Poisonous Plants.

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Chemical Studies upon the Vermeerbos, *Geigeria Aspera* Harv. I. Isolation of a Bitter Principle "Geigerin."

By CLAUDE RIMINGTON and G. C. S. ROETS, Section of Chemistry, Onderstepoort.

"VERMEERSIEKTE", or vomiting disease of sheep, has been known for some considerable time. According to Steyn (1934) the first record of the disease was due to Brandford in 1877, but it was not until 1928 that definite proof was forthcoming that its occurrence is due to ingestion of certain species of the composite *Geigeria*. In this year, du Toit (1928) reported that vomiting was produced by the experimental feeding of infusions of *Geigeria passerinoides* to a cow. In addition, experiments carried out at Donderbosfontein in 1923 had definitely shown that sheep grazing on veld cleared of everything but *Geigeria passerinoides* would contract the disease. A sufficient amount or "concentration" of feeding appears to be necessary; the individual susceptibility of animals also varies between wide limits.

Prior to this successful experiment, numerous negative results had been obtained by officers of the Research Division and by others (for details see Steyn, 1934).

Vermeersiekte has been responsible for very heavy losses of sheep in certain seasons. Thus in 1930, in a small area of Griqualand West, comprising about 20 farms, over fifteen thousand sheep were lost within three months from vermeersiekte (Steyn, 1934). The parts of the country most affected are the North-Western Cape Province, Griqualand West, Western Orange Free State and certain parts of the South-Eastern Transvaal, but heavy losses from vermeersiekte are also sustained in South West Africa. The plants concerned are all species of the Genus *Geigeria* and experimental work has shown that of these, *Geigeria aspera* Harv. is the most poisonous, *Geigeria passerinoides* Harv. only about one-tenth as harmful, whilst *Geigeria Zeyheri* Harv. and *Geigeria pectidea* Harv. occupy an intermediate position, although as previously mentioned, district and season appear to influence the toxicity of the plants. It has definitely been shown that toxicity is greatest in the pre-flowering stage and is much reduced after heavy rain has fallen. Concerning the stability of the toxin on storing, some doubt still remains.

Prior to the present work, nothing was known concerning the chemical nature of the active principle.

The action of the plant appears to vary somewhat and Steyn (1930) has divided vermeersiekte into four different forms, namely: —

(*a*) The vomiting form.

(*b*) The form in which hoven is the most outstanding symptom.

(*c*) The stiff form,

(*d*) The paralytic form.

Different species of animals are affected usually in different ways, but an animal may exhibit at the same time one or more of the above forms of the disease. The cause of death may be either—

(*a*) asphyxia caused by aspiration into the lungs of large amounts of vomited ruminal contents,

(*b*) paralysis of the centre of respiration,

(*c*) exhaustion caused by the incessant vomiting and diarrhoea, or

(*d*) heart failure.

With regard to the cause of the vomiting, two possible explanations present themselves, namely that the affect is a central one, the toxic substance causing stimulation of the vomiting centre in the medulla oblongata or that the irritation is local upon the walls of the rumen and abomasum, the persistence of the stimulus eventually inducing vomiting. The undoubted nervous character of the stiff and paralytic forms of the disease, lends support to the former hypothesis rather than to the latter. Post mortem, an acute catarrhal gastero-enteritis is nearly always to be observed.

Rotational grazing combined with eradication of the plant is the only preventive measure which has so far met with any success.

In pursuance of the programme of poison-plant research at present being carried out at this Laboratory, consignments of *Geigeria passerinoides* were obtained in 1932 from Boetsap in Griqualand West, but proved to be only slightly toxic. Of the dried, ground plant, 4 kilos were required to produce symptoms in a sheep. Rabbits were found to be unsatisfactory as test animals; however, by working with sheep it was definitely shown that the active principle is not extracted from the plant by cold water or by continuous extraction with hot petroleum. It is, however, removed by 96 per cent. alcohol and by exhaustive extraction of the plant with boiling ether.

In 1934, material of *Geigeria aspera* was obtained from Vereeniging and with it the work performed which is reported in the present paper. Since the quantity required for a single lethal dose was very considerable (in excess of 4 kilos for a sheep) the principle was adopted of purifying the toxic ether extract in which a substance was present giving a positive reaction with Wagner's reagent and consequently considered to be alkaloidal in type. This substance was isolated and shown to be a bitter principle. The yield is very small (average about 0·06 per cent. on weight of dried plant) and pharmacological tests have shown that it is not the substance producing vermeersiekte although it does not seem to be entirely devoid of toxic properties. 15 gm. given to a sheep in three successive doses over 2 days was without effect but in tests upon cats it proved positive, the animals vomiting continuously for about 2 days, after having received doses of 1 gm. by mouth. These experiments are fully reported later in this paper, and would seem to indicate that the substance in question exerts to a slight degree the same pharmacological action as does the vermeersiekte-producing principle. Its isolation and chemical examination are reported in the present communication.

ISOLATION OF THE BITTER PRINCIPLE.

Working upon the hypothesis that the material giving the positive iodine reaction was a base, ether extracts of the plant were shaken repeatedly with dilute acid and then, after filtration and making alkaline by sodium hydroxide, carbonate or ammonia, the aqueous solution was again shaken with ether in the expectation of transfering the base to this solvent. The recovery, as judged by the intensity of the iodine reaction, was extremely poor. This suggested that the substance was labile towards alkalis.

In order to eliminate the use of alkali, an ether extract was then shaken with dilute hydrochloric acid, which took out the material fairly readily, and this solution then shaken with chloroform. The material being sought passed at once into the chloroform but not, as was expected, as a hydrochloride, since on testing the extract it was found to be chloride free. On evaporation of the chloroform, a pale yellow oil remained in which large rhombic crystals gradually appeared. The same crystals were deposited together with a little oil when an ether extract of the plant was decolorised by charcoal and allowed to evaporate to dryness at room temperature. By washing with benzene and then petroleum ether the bulk of the oil adhering to the crystals was removed and recrystallisation could then be effected by dissolving in chloroform, adding a slight excess of petroleum ether and setting the cloudy solution in the ice chest for some hours. Such crystals had a bitter taste and gave a positive reaction with Wagner's reagent but were free from nitrogen and were therefore not alkaloidal in character.

The method was gradually improved until finally the following technique was worked out which has been found to give consistently good results.

METHOD OF ISOLATION.

The dried, ground plant was extracted by ether in a Soxhlet apparatus until the solvent was no longer coloured. The extract was then concentrated until its volume represented about 1,400 c.c. per kilogram of plant material. This ethereal solution was shaken repeatedly in a large separatory funnel with 1 per cent. hydrochloric acid until the aqueous phase no longer gave a positive iodine reaction or only a very slight reaction. Through the acid solution, about 2·5 to 3 litres in volume, a stream of air was now drawn until the smell of ether could no longer be distinguished. A good decolorising charcoal (Kahlbaum) 5 to 7 grams per litre of solution, was then added, the mixture shaken and filtered into a large separatory funnel. The water-clear aqueous phase was now shaken with small quantities of chloroform until this solvent no longer removed material giving a positive iodine reaction. The pale yellow chloroform extract was allowed to evaporate, either spontaneously or by blowing over it a stream of air until all the solvent was removed. There remained a pale, straw-coloured, viscous oil with a pleasantly aromatic smell. On standing, this generally crystallised, but a more convenient procedure was to boil up the oily residue with successive quantities of water, decant these and boil again with a pinch of charcoal and filter whilst hot into centrifuge tubes. Upon cooling, the liquid deposited a voluminous crop of beautifully formed large rhombic crystals. Crystallisation was completed in the ice-chest and the mother liquors centrifuged off. They contained a small quantity of non-crystallisable acidic oily material which was removed by chloroform extraction and reserved for further investigation. In all, about 150 to 200 c.c. of water was required to dissolve the chloroform residue from an original kilogram of plant material. A small quantity of tarry impurity insoluble in water was discarded.

The crystals were washed on the certifuge by ice-cold water and dried in vacuo over sulphuric acid. The yield averaged 0·06 gm. per 100 gms. of dried *Geigeria aspera* material, but was found to vary somewhat in different batches. Thus, in one exceptional instance a yield of 0·248 per cent. was recorded, however, the figure was generally found to be between 0·05 and 0·10 per cent. There was some indication that the more toxic batches contained the larger proportion of this constituent.

PROPERTIES OF THE SUBSTANCE.

The material, as isolated, crystallised in large rhombs, often grouped together (see Fig. 1). It was colourless and odourless and exhibited a double melting point. At 78° partial liquefaction occurred but the material rapidly resolidified and when the temperature was raised, melted sharply at 189°. All melting points were observed on a Kofler electrically-heated microscope stage. There appeared to be loss of substance at the lower temperature and this was confirmed by weighing and elementary analysis. Apparently 1 molecule of water is lost at 78° but regained upon recrystallisation from aqueous or moist solvents.

The substance was readily soluble in chloroform and warm absolute alcohol, sparingly soluble in ether and insoluble in petroleum ether. In benzene and warm ethyl acetate it was slightly soluble.

Recrystallisation is most easily effected from water but was successfully accomplished from other solvents as described below. From determinations it appeared that the difference in solubility in water between 100° and 0° is 1·14 gms. per 100 c.c. and the order of solubility at room temperature is 0·3 gms. per 100 c.c. water. Solutions of the substance have an intensely bitter taste which is very persistant; its dust irritates the eyes and nasal mucous membranes.

Recrystallisation from chloroform was accomplished by dissolving the material in a small quantity of this solvent, adding petroleum ether until a slight turbidity persisted and setting the mixture aside in the ice chest. It sometimes happened that crystals formed having the melting points 65-70° and 169° (β form) together with the typical form melting at 78° and 189° (α form). When recrystallisation was effected from hot ethyl acetate the β form predominated or appeared exclusively. Upon recrystallisation from water, it reverted into the α form melting at 189°. That this difference was not due to hydration in the aqueous solvent and separation of anhydrous substance from the ethyl acetate was clearly proved since,

(1) the elementary analyses of the two products were identical,

(2) the β form suffered the same loss of weight, (6·4 per cent.) as did the α form on heating at 85-90°. It seems, rather, to be a case of isomerism. The following investigations fully substantiate this view.

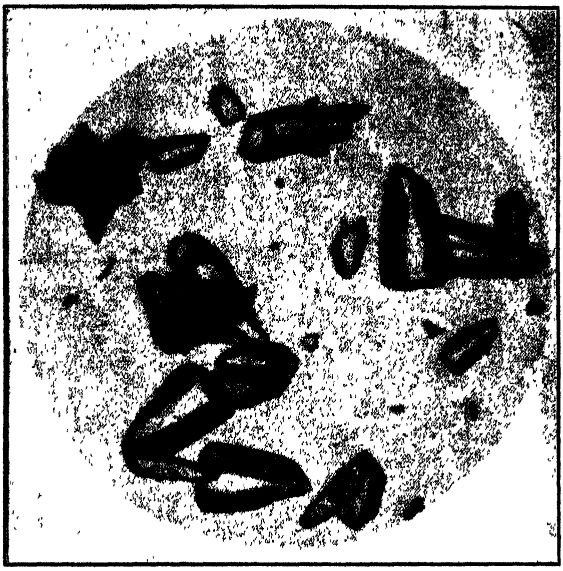
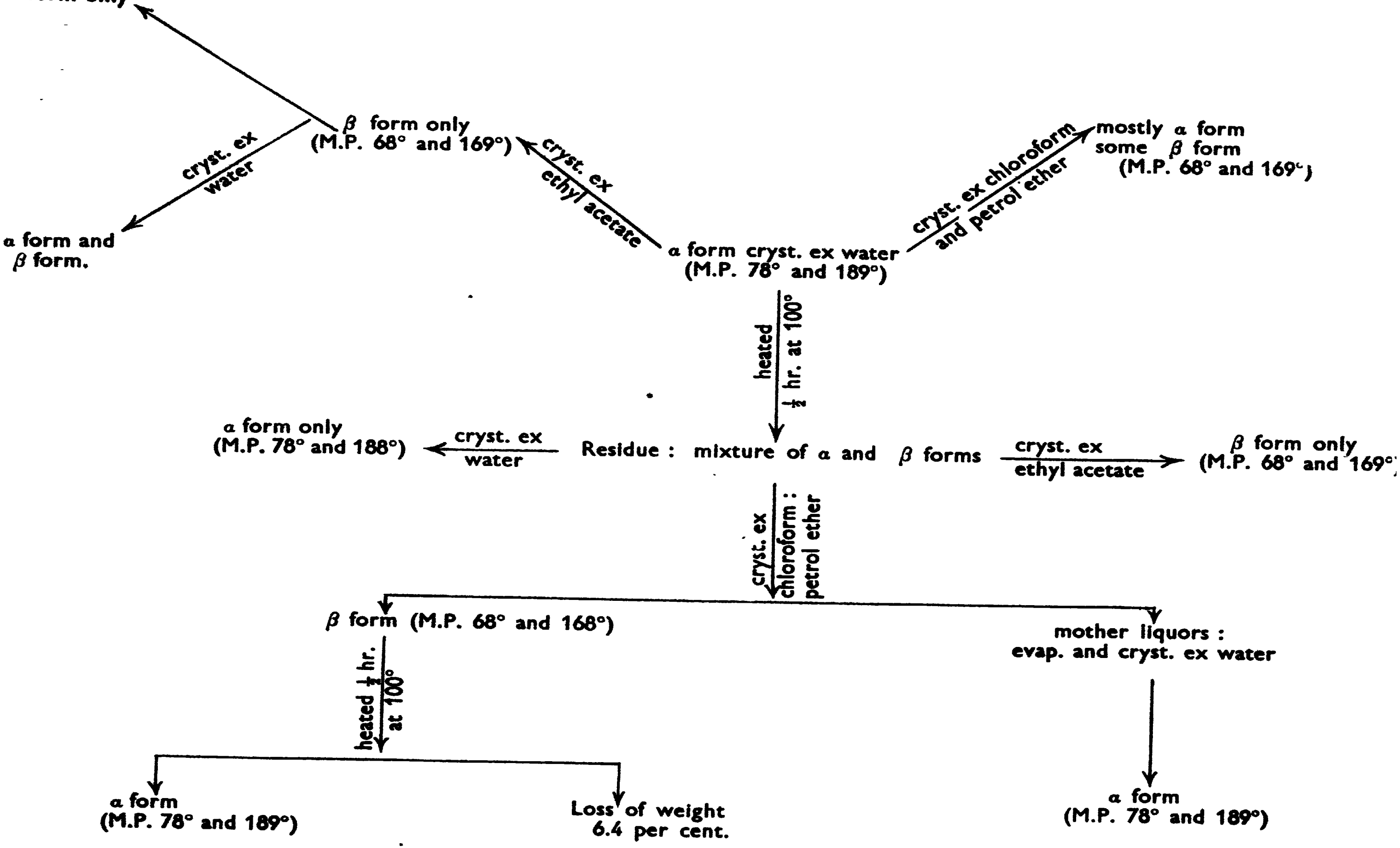


Fig. 1. Geigerin crystallised from water, ×63.

Isomerism of the Substance.

Upon heating some of the α form at 100° and taking samples at intervals for melting point determination, it was found that after the preliminary loss of approximately 6 per cent. of substance at 78° the residue melted sharply at 189°. One hour later, without further loss of weight, the melting point had altered, some crystals melting at 166° and some at 189°. Four hours later the weight was still constant but the melting point lay between 165° and 175°. In a similar manner the heating of the β form for periods of time extending from ½ to 2 hours yielded mixtures of this and the α form. The α form seems to be that more easily assumed.

EXPERIMENTS ILLUSTRATING ISOMERISM OF GEIGERIN.


α form only
β form only (M.P. 68° and 169°)
cryst. ex water
α form and β form.
cryst. ex ethyl acetate
α form cryst. ex water (M.P. 78° and 189°)
cryst. ex chloroform and petrol ether
mostly α form some β form (M.P. 68° and 169°)
heated ½ hr. at 100°
α form only (M.P. 78° and 188°)
cryst. ex water
Residue : mixture of α and β forms
cryst. ex ethyl acetate
β form only (M.P. 68° and 169°)
cryst. ex chloroform : petrol ether
β form (M.P. 68° and 168°)
mother liquors : evap. and cryst. ex water
heated ½ hr. at 100°
α form (M.P. 78° and 189°)
Loss of weight 6.4 per cent.
α form (M.P. 78° and 189°)

A difference in the optical rotatory power in different solvents, as recorded below, offers further evidence in favour of the hypothesis of isomerism. Before passing on to these figures, a summary is presented, in the chart above, of additional experiments carried out whilst investigating this isomerism. The organic solvents used were not specially dehydrated and there are suggestions that the very great affinity of the substance for the one molecule of water with which it crystallises enables it to assume this hydrated state even when crystallised, for example, from moist ethyl acetate.

A tendency is noticeable for the *β* form to separate from the organic solvents (this form also shows the double melting point, 68° and 169°, water being lost at the former temperature) although even from chloroform-petroleum ether, mixtures of *α* and *β* forms have been obtained and from alcohols of various strengths this is usually the case.

OPTICAL ROTATORY POWER.

This was determined in different solvents, using either *α* or *β* forms, in a Zeiss triple-field instrument with 2 dm. tube. The results may be summarised as follows:—

1. *α* form from water: solvent absolute alcohol.

$W = 0\cdot1010$ gm.
$V = 15$ c.c.
$\theta = -0\cdot82°$.

$$\therefore \quad \left[\alpha\right]^{22}_{D} = \frac{-0\cdot82 \times 100 \times 15}{2 \times 10\cdot1} = -60\cdot88°$$

2. *α* form from water: solvent 5 per cent. alcohol.

$W = 0\cdot0508$ gm.
$V = 16$ c.c.
$\theta = -0\cdot38°$.

$$\therefore \quad \left[\alpha\right]^{19}_{D} = \frac{-0\cdot38 \times 100 \times 16}{2 \times 5\cdot08} = -59\cdot85°.$$

3. *β* form from ethyl acetate: solvent absolute alcohol.

$W = 0\cdot0523$ gm.
$V = 15$ c.c.
$\theta = -0\cdot42°$.

$$\therefore \quad \left[\alpha\right]^{20}_{D} = \frac{-0\cdot42 \times 100 \times 15}{2 \times 5\cdot23} = -60\cdot23°.$$

4. α form from water: solvent chloroform.

$W = 0{\cdot}0502$ gm.
$V = 15$ c.c.
$\theta = -0{\cdot}285°$.

$$\therefore \left[\alpha\right]_{D}^{20} = \frac{-0{\cdot}285 \times 100 \times 15}{2 \times 5{\cdot}02} = -42{\cdot}58°.$$

5. β form from ethyl acetate: solvent ethyl acetate.

$W = 0{\cdot}0508$ gm.
$V = 15$ c.c.
$\theta = -0{\cdot}29°$.

$$\therefore \left[\alpha\right]_{D}^{20} = \frac{-0{\cdot}29 \times 100 \times 15}{2 \times 5{\cdot}08} = -42{\cdot}83°.$$

It is clear from the above that no matter whether the starting material is the α or β form of the substance, it exhibits the same optical rotatory activity in the same solvent. Two constants were observed, for absolute or dilute alcohol, in the mean $-60{\cdot}32°$ and for chloroform or ethyl acetate in the mean $-42{\cdot}71°$.

It is possible that this duality is related to the two isomeric configurations the substances may assume and may be an expression of keto-enol tautomerism (see discussion later). A similar irregularity of melting point was observed in the case of carolic acid (M.P. 112° and 132°) by Clutterbuck, Raistrick and Reuter (1935).

ELEMENTARY ANALYSIS AND MOLECULAR COMPOSITION.

Numerous analyses were performed upon specimens belonging to the α or β forms with consequently differing melting points but no difference whatever was observed in composition. All materials were nitrogen-free.

MICROANALYSIS.*

Material.	C.	H.	M. W.
α form crystallised from water, M.P. 78° and 189°.....	64·02	8·08	308
α form crystallised from water, M.P. 78° and 189°.....	63·99	7·47	298
β form crystallised from ethyl acetate, M.P. 68° and 169°	64·28	7·84	—
α form heated 1½ hours then crystallised from ethyl acetate, M.P. 65° and 169°..............................	64·00	7·82	—
α form heated 1½ hours then crystallised from water, then recrystallised from chloroform: petroleum ether, M.P. 65° and 168°..............................	64·08	7·90	—
$C_{15}H_{22}O_5$ requires..........................	63·84	7·80	282
Residue after heating to constant weight at 100°......	68·21	7·89	—
$C_{15}H_{20}O_4$ requires..........................	68·19	7·61	—
Loss of weight.......... 6·4 per cent.			
H_2O on $C_{15}H_{22}O_5$ requires 6·38 per cent.			

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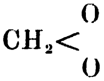
* All microanalyses by Dr. Backeberg, University of the Witwatersrand, to whom we wish to express our thanks.

Water vapour evolved on heating was identified by its action in restoring the blue colour to anhydrous copper sulphate. The molecular formula of the substance is therefore $C_{15}H_{20}O_4, H_2O$. Tests carried out for methoxyl CH_3O groups and dioxy-methylene  $CH_2<\begin{matrix}O\\O\end{matrix}$ groups proved negative. The substance was found to give certain colour reactions; these and other properties are listed below. Since neither bromine water nor bromine in chloroform was decolorised nor permanganate reduced in the cold, the substance would appear to be fully saturated, that is to say no ethylenic linkages $C = C$ are present. It gave no colour with tetranitromethane.

Colour Tests and other Reactions.

Wagner's iodine reagent	Amorphous precipitate.
Acid potassium iodide	Nil.
Phosphotungstic acid	White amorphous precipitate.
Picric acid	Yellow amorphous precipitate.
Sulphuric acid, concentrated	Nil.
Sulphuric acid, 50 per cent. by volume	Cerise colouration : same absorption spectrum as in hydrochloric acid.
Sulphuric acid added to a solution of the substance in acetic anhydride (Liebermann-Storch)	Intense transitory crimson colour.
Nitric acid	Nil.
Hydrochloric acid	Bright reddish-mauve colouration appearing slowly at room temperature, rapidly on warming. This colour though faint, is very characteristic. The solution exhibits three absorption bands with centres at 543, 506, and 466 (see Fig. 2) and has a faint greenish fluorescence.
Diazobenzenesulphonic acid	Nil.
Ferric chloride	Nil.
Bromine in chloroform	No decolorisation.
Potassium permanganate	No decolorisation in the cold.
Molisch test	Negative.
Fehlings solution	Slight reduction on prolonged boiling; more marked when material first boiled with dilute hydrochloric acid and then tested (see later in text).
Schiff's reagent	Nil.
Ammoniacal silver nitrate (Tollens)	No reduction.

Fig. 2. Action of HCl upon Geigerin. Absorption spectrum of cherry-red solution. Bands at 543; 506; 466 mμ.

PRESENCE OF A LACTONE GROUPING.

Although the substance is practically insoluble in cold water, it dissolves very slowly in boiling dilute alkali. The action of alcoholic alkali is more rapid and was used to demonstrate the presence of one lactone grouping in the molecule. Comparative resistance of a lactone to aqueous alkali but ready hydrolysis by alcoholic alkali is recorded by Hansen (1931) in the case of dihydroiso-alantolactone. 28·2 mgm. of material was dissolved in 5 c.c. of alcoholic potassium hydroxide (0·0804 N), a drop of phenol phthalein was added and the mixture immediately back titrated by decinormal hydrochloric acid. Alkali neutralised amounted to 0·25 c.c. There is thus no free carboxyl group present in the substance. A further 5 c.c. of alcoholic potash was added and the mixture back titrated after standing overnight at 37°.

5 c.c. alc. KOH is equivalent to 3·65 c.c. of 0·1086 N hydrochloric acid.

back titration required 3·0 c.c.
difference 0·65 c.c.
difference in preliminary titration 0·25 c.c.
∴ Amount of alkali neutralised by substance =
0·90 × 1·086 c.c. of N/10.
= 0·98 c.c.
Theory for 1 lactone group = 1·00 c.c.

In a second experiment, 28·2 mgm. of material dissolved in 5 c.c. of alcoholic potash was left for 1 hour at 37° and then back titrated. 1·08 c.c. n/10 was neutralised as against the theoretical value of 1·00 c.c.

A definite excess of acid was then added and after half an hour the mixture again titrated. No change was observed, indicating that no spontaneous reformation of the lactone ring takes place when the free acid is left in solution for this length of time.

OPTICAL ROTATORY POWER OF THE FREE ACID.

In preliminary experiments, the rotation was determined of the reaction mixture of the material and alcoholic potassium hydroxide before and after acidification. The same value was obtained in each case, thus:—

47·17 mgm. in 5 c.c. alc. potash for 3 hours at 37°.

Volume made up to 22 c.c. Rotation observed +0·48°.

$$\left[\alpha\right]_{D}^{21} = \frac{+0\cdot48 \times 100 \times 22}{2 \times 4\cdot717}$$
$$= +111\cdot9^{\circ}.$$

In another case, 50 mgm. was left in 5 c.c. alc. potash overnight at 37°. The solution was diluted to 15 c.c. Rotation observed +0·70°. A drop of concentrated acid was now added to bring to acid reaction but the rotation remained unchanged.

$$\therefore \left[\alpha\right]_{D}^{20} = \frac{+0{\cdot}70 \times 100 \times 15}{2 \times 5{\cdot}0}$$
$$= +105^{\circ}.$$

The free acid and its salts are therefore strongly dextrorotatory (see preparation of Geigeric acid, later).

Considering the abovementioned properties of the substance isolated from *Geigeria aspera*, it is considered that it belongs to the class of bitter principles. No substance with identical properties has been described in the literature and for this reason it is proposed to assign to it the name *Geigerin*.

There is, however, a record of the isolation of a crystalline substance from *Chrysanthemum cinerariaefolium* by Thoms in 1891 (see Klein, 1933) and from the data available it appeared to show certain points of similarity with Geigerin. Thus the melting point is recorded as 188-189° and the formula given as $C_{34}H_{44}O_{10}$, although with some reserve. Moreover the solubilities appear to be very similar to those of Geigerin and this substance, which Thoms designated "Pyrethrosin", also gave a reddish-violet colouration on warming with hydrochloric acid.

Unfortunately, attempts to obtain the journal in which Thoms' paper appeared were unsuccessful both in South Africa and by purchase from Europe and it would not have been possible to extend the comparison of the two substances any further had it not been for the kindness Professor Th. Sabalitschka, of the Pharmakologisches Institut, Berlin, who very kindly made a copy of Thoms' article (Berichte d. Deutsch. Pharmazeut. Ges. 1, 241, 1891) which he sent to one of us (C.R.). I wish to express my sincere thanks to Professor Sabalitschka.

Thoms does not record the optical activity of his substance Pyrethrosin, nor was a molecular weight determination carried out. From the general description, however, the colour reaction with hydrochloric acid and the production of a substance reducing Fehling's solution when the material is boiled with dilute acid, a substance which Thoms proves is not a sugar, there remains little doubt that "Pyrethrosin" and "Geigerin" are identical. The true molecular formula is $C_{15}H_{22}O_5$ not $C_{34}H_{44}O_{10}$. Thoms' Pyrethrosin may not have been quite pure. He used only organic solvents for crystallisation and it is difficult under these conditions to eliminate traces of sticky or tarry materials. The occurrence of one and the same bitter principle in *Chrysanthemum cinerariaefolium* and *Geigeria aspera* is of great interest. Thoms made no pharmacological test upon his material other than to show that it was devoid of insecticidal properties. Both plants belong to the family *Compositae* and it would be of interest to examine South African *Geigeria* species for the presence of the insecticidal pyrethrin oils.

Absence of Hydroxyl Groups – OH from the Geigerin Molecule.

In order to establish the presence or absence of hydroxyl groups in Geigerin, attempts were made to acetylate the substance, as follows, but without success. In each case the material was recovered unchanged.

(i) 50 mgm. substance was dissolved in 2 c.c. of chloroform, 0·2 c.c. of acetic anhydride added and the mixture left overnight. Petroleum ether was then added, causing the separation of a crystalline material in small stellate clusters of prisms. These proved to be unchanged Geigerin with M.P. 73° and 167-9°.

(ii) 50 mgm. substance was dissolved in 1 c.c. of pyridine, 0·15 c.c. of acetic anhydride added and the mixture left for several days. On adding petroleum ether, unchanged Geigerin crystallised out, M.P. 76° and 168-9°.

(iii) 50 mgm. of substance in 2 c.c. chloroform plus 0·5 c.c. acetic anhydride was refluxed for 8 hours. On addition of petroleum ether to the slightly yellow solution, unchanged Geigerin crystallised out, both α and β forms being present. M.P.'s. 74° and 166° also 77° and 187-8°.

Boiling with acetic anhydride. and sodium acetate led to complete destruction and tar formation.

Since certain substances may contain hydroxyl groups which are difficultly acetylated but react smoothly with phenylisocyanate to form phenylurethanes, an experiment was conducted as follows.

(iv) 50 mgm. of substance were dissolved in 2 c.c. of chloroform, 0·75 c.c. of phenylisocyanate added and the mixture set aside for several days. Some squarish crystals appeared but these proved to be diphenylurea with M.P.. 258°. To the mother liquor, petroleum ether was added and the crystals which separated removed; they proved to be unchanged Geigerin M.P. 78° and 168° also some 187° (α form).

It may be concluded, therefore, that hydroxyl groups are absent from Geigerin.

Demonstration of the Presence of One Ketonic >CO Group.

To 30 mgm. of substance dissolved in 15 c.c. of hot water was added 3 c.c. of hydrochloric acid and then 1·5 c.c. of hot Brady's reagent—a solution of 0·5 gm. of 2:4 dinitrophenylhydrazine in 30 c.c. of normal hydrochloric acid. The colour deepened and almost at once the precipitation in microcrystalline form commenced of a deep orange-red substance. This was separated by centrifuging, washed with 2 normal hydrochloric acid and then with water and finally recrystallised from hot 60 per cent. alcohol. It separated in rosettes of red plates M.P. 250-2°.

Microanalysis.

	C.	H.	N.
Found..........	56·58	5·70	12·80
$C_{21}H_{24}N_4O_7$ requires........	56·72	5·45	12·61

ABSENCE OF AN ISOPROPYL SIDE CHAIN.

In order to test for the presence of an isopropyl side-chain, a group occurring in several terpenes and bitter principles, 0·10 gm. Geigerin, dissolved in a little glacial acetic acid, was placed in a micro-kjeldahl distillation apparatus and a current of steam passed through whilst an oxidation mixture of chromic acid in glacial acetic acid was added slowly during the course of 2 hours.

The distillate was neutralised and again distilled, the first 20 c.c. being tested for acetone. Brady's reagent gave no turbidity and all colour tests were negative. The residual oxidation mixture was placed in the ice-chest when large colourless rhombic crystals (60 mgm.) separated. These were recrystallised from water and shown to be unchanged Geigerin. M.P. 78° and 168-9°.

$$\left[a\right]^{21}_{D} = \frac{-0\cdot40 \times 100 \times 16}{2 \times 5\cdot03}$$
$$= -63\cdot63^\circ.$$

Alcoholic KOH neutralised by 15 c.c. of this solution during 12 hours at 37° = 1·76 c.c. N/10.

Theory requires 1·67 c.c.

The lactone grouping was therefore still intact and in every respect the substance proved to be identical with Geigerin. No other product could be isolated from the reaction mixture.

Geigerin, therefore, contains no isopropyl side chain and the whole molecule would appear to be fairly stable towards oxidising agents.

ZINC DUST DISTILLATION.

In preliminary experiments, Geigerin was distilled with zinc dust in a glass tube heated to about 400° and in presence of a current of hydrogen. The reaction products were dark, pleasant-smelling oils, dissolving in ether with a bluish fluorescence. Fractionation from absolute alcohol was attempted but insufficient material was obtained in a pure state for analysis.

It appeared that these oils contained hydro-aromatic hydrocarbons. They gave an intense red colour with vanilin-hydrochloric acid. A distillation was therefore carried out at a higher temperature, 700°, and the hydrogen stream stopped after all air had been displaced from the apparatus. There resulted a white crystalline distillate mixed with a little yellow oil. The crystals were easily drained and proved to be naphthalene. They sublimed easily, had M.P. 79°, yielded a picrate crystallising in needles with M.P. 150° and possessed the characteristic smell of naphthalene.

POTASH FUSION: PRELIMINARY EXPERIMENTS.

A potash fusion was carried out in a nickel crucible at 280°-300° for 3 hours using 1 gm. of Geigerin and 15 gm. of potassium hydroxide. The melt was almost completely soluble in water. It was acidified and steam-distilled, yielding an acid distillate possessing a rancid, butyric-like smell. The sodium salt prepared by neutralisation proved to be gelatinous whilst the acid itself could not be obtained in the solid state; the material was therefore converted into the insoluble, amorphous silver salt for analysis Yield: 33 mgm.

Microanalysis.

	C.	H.	Ag.
Found	32·64	4·65	51·30

These figures agree most nearly with $C_6H_9O_2Ag$. Capronic acid, $C_6H_{12}O_2$, possesses an amorphous silver salt but a copper salt which crystallises in a characteristic manner. The acid obtained from Geigerin did not yield these crystals.

From the non-volatile fraction, other rancid-smelling oily acids were obtained but in no case could any individual be identified with certainty, hence the experiments were discontinued. The absence of any easily crystallised solid acid was noteworthy and since many of the materials isolated gave a cherry-red colour reaction with vanilin-hydrochloric acid, similar to that obtained with the naphthenic acids, the suspicion was engendered that the products might belong to the polymethylene series.

Only in one instance was a well-crystallised sodium salt obtained and this and the corresponding silver salt on analysis appeared to indicate a parent dibasic acid with the formula $C_7H_{12}O_8$. What structure such an acid might have it is impossible to hazzard, but since the analytical figures agreed fairly well and these experiments are only of a preliminary nature the data will be recorded.

	C.	H.	Na.
Sodium salt. Found.........	30·85	4·02	17·10
C_7H_1 O Na_2 requires.........	31·34	3·73	17·16
Silver salt. Ag. found.......	—	48·62	—
$C_7H_{10}O_8Ag_2$ requires.........	—	49·29	—

Geigeric Acid.

The free acid of which Geigerin is the lactone was prepared by dissolving 0·5 gm. Geigerin in 25 c.c. of approximately 0·1 normal alcoholic potassium hydroxide and after 24 hours at 37° acidifying the solution with the requisite quantity of hydrochloric acid. Upon shaking with ether, after dilution, the free acid passed over into the ether phase which was washed and evaporated to dryness, the residue being crystallised from hot petroleum ether in which it was sparingly soluble. The acid separated in beautiful, elongated flat-plates (see Fig. 3) and had M.P. 201–3°. It was only moderately soluble in chloroform, more soluble in absolute alcohol, which solvent was used for the determination of optical rotatory power. 30 mgm. substance in 15 c.c. absolute alcohol had rotation +0·42°.

$$\left[\alpha\right]^{28}_{D} = \frac{+0\cdot42 \times 15 \times 100}{2 \times 3} = +105^{\circ}.$$

Micro-analysis.

	C	H.
Found....................	63·79	7·92
$C_{15}H_{22}O_5$ requires................	63·84	7·80

It is proposed to designate this substance "Geigeric acid"

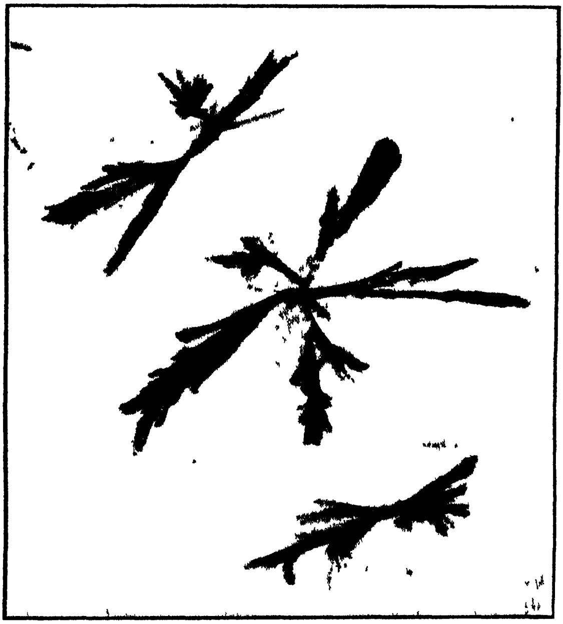


Fig. 3. Geigeric acid, M.P. 201-3°, ×70.

The Action of Hydrochloric Acid upon Geigerin.

The colour reaction given by Geigerin with hydrochloric acid has already been mentioned. There develops in the cold, more readily on warming, a faint cherry-red colour which is stable for a considerable period but very gradually assumes a browner tint. Solutions of hydrochloric acid more concentrated than 10 per cent. by weight yield the colour readily but with weaker solutions only a very faint pinkish-mauve colour is gradually formed. Chloroform does not extract this colour, but it is discharged at once by dilution with water and chloroform is then capable of extracting unchanged Geigerin from the solution. Similarly, if a concentrated solution of Geigerin in 20 per cent. hydrochloric acid be diluted largely and placed in the ice chest, crystals of unchanged Geigerin slowly separate, but Geigerin is so much more easily soluble in dilute hydrochloric acid than in water that the yield is far from quantitative.

The cherry-red solution in 20 per cent. hydrochloric acid exhibits a well-marked absorption spectrum, the centres of the bands being at 543; 506 and 466 mμ respectively, the relative intensities being in descending order 543; 466; 506 (see Fig. 2). It is noteworthy that Vermeeric acid, the active principle of the Vermeerbos, to be described in the second paper of this series, gives the colour reaction very vividly, the positions of the bands being almost identical in the two cases, although the colour tint varies somewhat.

Prolonged action of hydrochloric acid undoubtedly causes some alteration in the structure of Geigerin. The following experiment was therefore carried out in an attempt to discover the nature of this change.

0·1 gm. of Geigerin was refluxed for 1 hour with 5 c.c. of 20 per cent. hydrochloric acid, the brown solution which possessed a pleasant aromatic smell was diluted, neutralised with sodium hydroxide and concentrated by distillation; the distillate gave no precipitate with Brady's reagent. The main solution was shaken with ether which extracted only a very small quantity of resinous material. It was then acidified and again extracted with ether which removed a pale yellow acidic oil. As all attempts to induce crystallisation failed, this material was esterified with diazomethane and the methyl ester crystallised from hot petroleum ether in which it was sparingly soluble. It was obtained in aggregates of very beautiful colourless, flattened prisms having pointed ends. The M.P. was 98·5°.

Micro-analysis.

	C.	H.	CH_3O.
Found......	64·96	8·11	10·32
$C_{15}H_{21}O_4$ (CH_3O) requires.....	64·82	8·17	10·47

The parent acid therefore possesses the same formula $C_{15}H_{22}O_5$ as Geigeric acid, showing that the lactone ring must have been opened during the reaction. Whether this acid and Geigeric acid are identical or isomeric, as is thought to be more probable, has not as yet been determined.

There is some evidence which suggests that the ketonic group yielding the 2:4 dinitrophenylhydrazone of Geigerin, described earlier, may form a part of some tautomeric grouping in the molecule, since the presence of hydrochloric acid appears to be essential for the formation of derivatives of this function. Thus, an attempt was made to prepare the dinitrophenylhydrazone by refluxing Geigerin with a slight excess of 2:4 dinitrophenylhydrazine in alcoholic solution but no reaction took place. Similarly an attempt to prepare a semicarbazone failed.

Should Geigerin exhibit keto-enol tautomerism, this might explain the two melting points observed with the pure substance, but the failure to detect an hydroxyl group by acetylation must be recalled. Comparison with the behaviour of carolic acid may prove significant in this connection (Clutterbuck, Raistrick and Reuter, 1935). On treatment with Brady's reagent, carolic acid gives an immediate precipitate of a mono-dinitrophenylhydrazone by virtue of the ketonic group in its side chain. The tetronic acid ring structure, however, possesses, in addition, an oxygen atom which shows keto-enol tautomerism and this more slowly reacts with a further equivalent of the reagent to form a bisdinitrophenylhydrazone. Attempts to acetylate carolic acid in its enol form by boiling with acetyl chloride failed, only unchanged starting materials being recovered.

OXIDATIVE DEGRADATION OF GEIGERIN.

Geigerin does not decolourise cold permanganate solution but is fairly readily oxidised at the boiling temperature. 1 gm. Geigerin in 20 c.c. of water plus 1 gm. sodium carbonate required 82 c.c. of 2·5 per cent. potassium permanganate solution, added in small quantities at a time, before the reaction slowed up. The resultant mixture was filtered, affording a pale yellow liquid which gave a yellow precipitate with Brady's reagent. It was therefore concentrated by distillation (whilst still alkaline) and the distillate tested but with negative result; no substance reacting with Brady's reagent was present. The main solution was then extracted very thoroughly by ether, this solvent slowly but completely removing from the alkaline liquid the material giving the dinitrophenylhydrazone. Upon evaporation, the ether left a pale oil with a peculiar cucumber-like smell. It was fairly easily soluble in water and was found to give the following reactions:—

Schiff's reagent	Fairly rapidly +.
Fehling's solution....................	Only reduced on boiling for 1 minute.
Tollen's reagent ($AgNO_3 : NH_4OH$)..	—
Jean's test (Phenylhyrazine + Nitroprusside)	slowly brown.
m-dinitrobenzene + alkali...........	yellow only.
Thiophene + H_2SO_4 test............	+ cherry red.

Upon acidifying and adding Brady's reagent, a 2:4 dinitrophenylhydrazone precipitated and was separated off, washed and crystallised from hot dilute alcohol. It crystallised in yellow plates with M.P. 158-161° and proved to be identical with the derivative of acetaldehyde, thus:—

Micro-analysis.

	N.
Found..............................	24·84
$C_8H_8N_4O_4$ requires..............................	25·00

Mixed with acetaldehyde 2:4 dinitrophenylhydrazone of M.P. 159-161°, it had M.P. 159-161°.

This result was somewhat surprising, but it was noticed that of the colour tests listed above, only those were positive (and similar to acetaldehyde) which required the addition of acid to the solution. The substance isolated from the oxidation mixture clearly could not be identical with acetaldehyde since it could not be distilled but it was suspected that it was some material which affords acetaldehyde when treated with acids. (Compare the acetals which are stable to hot alkalies but yield aldehyde immediately on addition of cold dilute acids.)

This supposition was proved to be correct in the following way: To some of the substance was added 2 normal sulphuric acid and the mixture was gently distilled affording a distillate giving all the tests for acetaldehyde and from which acetaldehyde 2:4 dinitrophenylhydrazone was prepared.

Tests on Distillate.

Schiff's reagent	+
Fehling's solution	Reduced on boiling.
Tollen's solution	+
Jean's test	+ red.
m-dinitrobenzene + alkali	+ mauve.
Iodoform	+
Thiophene	+

Micro-analysis.

		N
2:4 dinitrophenylhydrazone M.P. 158-160°.	Found	25·33
Acetaldehyde dinitrophenylhydrazone requires		25·00

The mixed M.P. was 159-160°.

It was subsequently found, as reported in the following paper, that Vermeeric acid, on oxidation by means of alkaline permanganate, also yields a similar material which when treated with dilute acid liberates acetaldehyde.

The alkaline aqueous solution remaining after having removed, by ether extraction, all the material reacting with Brady's reagent, was acidified and again exhaustively extracted with ether which now removed a strongly acidic substance. This was converted into the sodium salt and the latter, after washing with acetone, dissolved in absolute alcohol and precipitated by ether. There was obtained 0·42 gm. of a nearly colourless amorphous powder. Attempts to crystallise the free acid from this small quantity failed; however an analysis was performed and after methylation with diazomethane in ethereal solution the methyl ester was obtained crystallising from hot petroleum ether, in which it is sparingly soluble, in short hexagonal prisms, M.P. 115-6° (see Fig. 4). On repetition, the acid itself was obtained crystallising in six-sided prisms, M.P. 200-1°, $[\alpha]_D + 90°$. Analysis indicated the formula $C_{10}H_{14}O_4$.

Micro-analysis.

	C.	H.
Methyl ester. Found........	60·55	6·95
$C_{10}H_{14}O_4$ requires........	60·60	7·07

The substance appeared to react slowly with Brady's reagent forming a yellow precipitate.

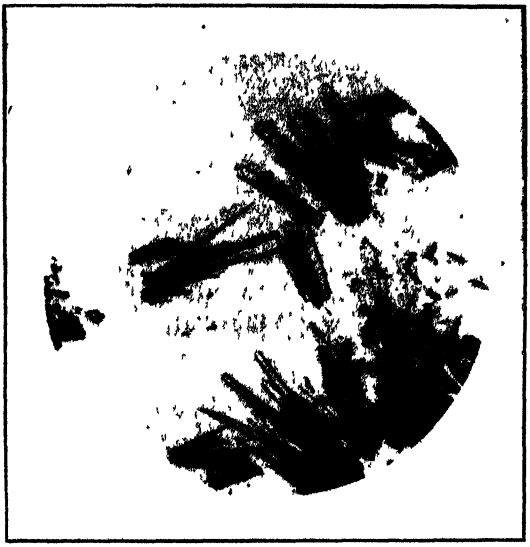


Fig. 4. Methyl ester of acid obtained by alkaline permanganate oxidation of Geigerin, M P 115-6°, ×66.

These experiments must be regarded as purely preliminary; further work awaits the accumulation of sufficient starting material. In general, the chemical work reported above supports the idea that Geigerin may be made up of hydroaromatic or polymethylene ring systems but certainty upon this point can only be obtained by the isolation of degradation products in sufficient quantity to allow of their transformation into known substances. The object of the present communication is to record the isolation of the lactone Geigerin from *Geigeria aspera* and to describe its reactions and properties. The question of its exact chemical structure will be dealt with in another communication at some future date.

PHARMACOLOGICAL EXPERIMENTS

A cat (1,200 gm.) was given 0·1 gm. Geigerin dissolved in 3 c.c. of 30 per cent. alcohol subcutaneously. The animal looked ill next day and would only drink a little milk; however, complete recovery eventually took place.

About six months later the same animal was given 1 gm. of Geigerin suspended in water by stomach tube at 12.30 p.m. Symptoms developed as follows:—

12.47 p.m. Vomited.
12.55 ,, Vomited again.
1.5 ,, Vomited again.
2.2 ,, A severe attack of retching, which brought up only a very small quantity of fluid together with a tape-worm about 5·5 inches in length
2.40 ,, Further retching.

The animal remained very ill throughout the day, but drank a little milk. Intense salivation and retching occurred at intervals. Next day it refused to eat and looked very ill. The following day another retching attack was observed at 11.30 a.m. and a considerable amount of fluid containing mucous was seen in the bottom tray of the cage suggesting that vomiting had occurred unobserved during the night. The animal was killed. The post mortem examination revealed a severe gastro-enteritis confined more particularly to the fundus region of the stomach. The trachea and glottis were normal. The liver showed slight fatty infiltration. The urine in the bladder was free from haemoglobin.

On a subsequent occasion, the same dose (1 gm.) was given in the same manner to a 2·1 kilo cat. It remained quiet and listless all day. Next morning vomited material was observed in the cage. The animal refused food and looked very ill. On the following day it appeared somewhat improved and was killed for post mortem examination. A slight mucous catarrh was seen in the stomach and small intestine, otherwise the appearance of all organs was normal.

A rabbit (1·9 kilos) which received 0·25 gm. Geigerin by stomach-tube exhibited no symptoms. A sheep was drenched with a total quantity of 15 gm. of Geigerin given in doses of 5 gm. at intervals over 2 days. It remained normal. These preliminary pharmacological tests suggest that cats are the most suitable test animals to be used. Geigerin would appear to have little or no action upon rabbits but to be not entirely devoid of vermeersiekte producing properties (gastric irritation) as judged by its effect when dosed to cats. It is hoped to continue this aspect of the work at some later date.

SUMMARY.

1. There has been isolated from toxic consignments of *Geigeria aspera*, Harv. a neutral lactonic bitter principle to which the name *Geigerin* has been assigned. This substance, though not the true toxic principle, would appear to be not altogether devoid of pharmacological properties.

2. Geigerin has the formula $C_{15}H_{20}O_4$ and crystallises from water or moist organic solvents with 1 molecule of water of crystallisation. It exhibits isomerism, the melting points of the two varieties, when anhydrous, being 169° and 189° but preliminary softening with liberation of water from the hydrated prisms is observed at about 68° and 75° respectively. No matter which isomer is used, the same optical activity is exhibited in the same solvent, the constants being for absolute or dilute alcohol $\left[\alpha\right]_D^{20} = -60 \cdot 32^\circ$ in the mean and for chloroform or ethyl acetate $\left[\alpha\right]_D^{20} = -42 \cdot 71^\circ$ in the mean.

3. There are some grounds for believing the isomerism to be due to keto-enol tautomerism, although no hydroxyl group could be detected by acetylation under various conditions.

4. Geigerin forms a 2:4 dinitrophenylhydazone in the presence of hydrochloric acid, but attempts to prepare derivatives of the ketonic function in the absence of an acid failed.

5. Potassium permanganate is not decolorised by the substance in the cold neither can any acetone be recovered after the action of chromic acid under the proper conditions for the oxidation of isopropyl side-chains to acetone. Such a group is therefore not present in Geigerin.

6. Cold hydrochloric acid of concentration over 10 per cent. affords a very striking colour reaction with Geigerin. The substance dissolves easily, forming a cherry-red solution which shows absorption bands at 543, 506 and 466 mμ. In time, the colour becomes more brown in tint.

7. Geigerin is a monolactone and the corresponding acid, *Geigeric acid*, has been prepared and found to crystallise in plates with M.P. 201-3° and $\left[\alpha\right]_D^{28} = +105^\circ$.

8. When Geigerin is boiled for 1 hour with concentrated hydrochloric acid the cerise colour changes to brown and from the reaction mixture an acid was isolated in the form of its methyl ester melting at 98·5°.

9. From results reported in the following communication, it would appear that Geigerin is closely related, chemically, to the active principle of the plant which causes vermeersiekte. Preliminary degradation experiments indicate that on potash fusion a mixture of liquid rancid-smelling acids is produced, and certain reactions suggest that these may belong to the polymethylene series of compounds.

10. On distillation at low temperature with zinc dust, fluorescent, oily aromatic substances are produced. At 700° naphthalene is formed.

11. When Geigerin is oxidised by hot alkaline potassium permanganate, there arises an acidic fraction from which the methyl ester of an acid, probably in $C_{10}H_{13}O_3$ (OCH_3), was isolated in crystalline form, M.P. 115-6°, and also a neutral, non-volatile substance soluble in ether and slightly soluble in water, which on treatment with dilute mineral acid in the cold at once liberates acetaldehyde. The exact nature of this material awaits further investigation, but attention is drawn to the analagous behaviour of the acetals.

12. It would appear from the literature that Geigerin is in all probability identical with the substance extracted by Thoms from *Chrysanthemum cinerariaefolium* in 1891 and named by him " Pyrethrosin ". His formula for this substance $C_{34}H_{44}O_{10}$ was put forward with some reserve.

13. Preliminary pharmacological experiments upon rabbits, sheep and cats are reported.

ACKNOWLEDGEMENTS.

We wish to express our thanks to Mr. T. du Toit, of the farm Skaapplaas, and through him to Vereeniging Estates, Limited, for their kindness in procuring *Geigeria aspera* material for the purpose of this work.

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Chemical Studies upon the Vermeerbos, *Geigeria Aspera*, Harv. II. Isolation of the Active Principle, "Vermeeric Acid."

By CLAUDE RIMINGTON, G. C. S. ROETS and DOUW G. STEYN, Sections of Chemistry, Pharmacology and Toxicology, Onderstepoort.

IN the preceding communication (Rimington and Roets, 1936) the isolation has been described of a lactonic bitter principle, $C_{15}H_{20}O_4$, named "Geigerin" from the Vermeerbos *Geigeria aspera*, Harv. (Compositae). The present paper deals with the isolation from this plant of the active principle causing vermeersiekte, or vomiting disease, and its characterisation as a dibasic acid $C_{18}H_{28}O_7$ which readily passes over into the dilactone $C_{18}H_{24}O_5$. It is proposed to call the free acid "Vermeeric acid" and its dilactone "Vermeerin". Both substances show certain marked points of similarity with Geigerin, and it is probable that their structures are closely similar. Empirically, Vermeerin differs from Geigerin by the elements C_3H_4O but it still gives the colour test with hydrochloric acid (reddish solution with absorption bands in the visible spectrum) although with much less intensity than does the latter and it also affords on alkaline permanganate oxidation, a substance yielding acetaldehyde on treatment with cold dilute acids.

During the course of the investigation there were also isolated two substances which appear to be flavones and which were only separated from Vermeeric acid with difficulty. They are not considered to be in any way related to the toxic principle.

Pharmacological trials have shown that rabbits are entirely unsuited for the testing of vermeersiekte activity. Frogs, on the other hand, would appear to offer considerable possibilities, especially since the doses required to cause paralysis and death are of the order of a few milligrams only. Quantities of 10 to 15 gm. of vermeeric acid given orally to sheep cause death from acute vermeersiekte within 5 to 24 hours. The pharmacological examination of this substance is reserved for a future publication.

PRELIMINARY TESTS.

Quite early in these experiments, when attention was being directed towards the isolation of the lactone Geigerin, it was noticed that the final fractions contained a considerable quantity of an acidic oil with a pleasantly aromatic odour. Dosing of this material or the ether extract of 80 gm. of dried plant to rabbits was without effect although frogs which received about 50 miligrams injected into the dorsal lymph sac suffered from paralysis and respiratory distress, death usually occurring fom asphyxia within an hour. The neutral fractions of the extracts were almost devoid of activity.

Experiments upon sheep showed that the active principle could be extracted by boiling ether and transferred, by shaking, to dilute aqueous sodium carbonate solution, thus: 1·5 kilos of dried plant (Vereeniging Estates, 1st Lot; effective dose for sheep ±750 gm.) were extracted by ether and the extract shaken with 1 per cent sodium carbonate solution. The ether soluble residue was dosed with negative result. The clear, brown aqueous liquid was then acidified and shaken with chloroform which removed a pale yellow acidic oil. This was given to a sheep per os with the following result. Dosed 3 p.m.:—

Next morning, 9 a.m.	Listless, not eating; temperature 101°.
11.30 a.m.	Walking with a stiff gait; temperature 103°.
2 p.m.	Prostrate; forced respiration. Pulse 160, hard and jerky. Some signs of nervous hypersensibility.
2.15 p.m.	Died.

Post mortem.—Gastro-intestinal inflammation, ulcers in the omasum associated with particles of a brown material. Heart flabby with haemorragic patches covering the left auricle. Liver friable, and somewhat fatty with central stasis. Kidney medulla slightly engorged. Some fresh ingesta found in the upper portion of the trachea.

In a repetition of this experiment, the sodium carbonate solution was shaken repeatedly with fresh chloroform in order to remove completely all non-acidic material since Geigerin is appreciably soluble in aqueous solutions. There was recovered in this way 6·2 gm. of Geigerin crystals, but the residual acid fraction weighing 15·5 gm. was still very toxic, the test sheep dying within 5·5 hours after dosing. A small chloroform-insoluble, dark, amorphous material which separated when the carbonate solution was acidified was dosed separately but proved to be inactive.

Various methods of purification of the active fraction were resorted to, such as high vacuum distillation, but a certain degree of lability of the principle, especially at high temperatures, led to rapid loss of toxicity.

At this stage, it was noticed that as the solvent evaporated, chloroform solutions of the acidic oil deposited a small quantity of a yellow material which was practically insoluble in chloroform when the mixture was stirred again with fresh solvent. In this way a further purification was achieved and on an accumulated sample of the active chloroform-soluble material certain tests were carried out as follows:—

Toxicity.—10 gm. dosed to a sheep caused death from typical acute vermeersiekte within 24 hours, the onset of symptoms being noticed about 8 hours after dosing.

7 gm. caused symptoms of stiffness and vomiting but the animal made a gradual recovery.

HCl Colour Reaction.—With concentrated hydrochloric acid a brownish-red colour developed slowly but more rapidly on warming and the solution exhibited absorption bands at approximately 542, 502 and 465 mμ. The bands were less distinct and the colour more brownish than is the case with Geigerin.

Optical Rotation.—A 0·52 per cent. solution in alcohol was optically inactive (2 dm. tube).

Equivalent by Titration.—0·3564 gm. of the oil dissolved in alcohol and titrated to phenol phthalein required 12·23 c.c. of 0·1 N sodium hydroxide. The sodium salt dissolved in alcohol and precipitated by ether was analysed with the following result:—

Micro-analysis.—

		Na
Crude Na salt of toxic acid.	Found	10·59

It can be mentioned that the calculated value required by the formula $C_{18}H_{26}O_7Na_2$ (based on results to be described later) is 11·50.

SEPARATION OF TWO FLAVONES.

A modification introduced into the procedure for the preparation of the toxic fraction resulted in a very much more complete and easy separation of the yellow material referred to above.

An ether extract of 1·5 kilos of plant powder was treated in the usual way, but in order to prevent emulsification and accelerate the separation of the phases when the sodium carbonate solution was shaken with chloroform, an addition of about 50 gm. of sodium chloride was made. This allowed of much quicker working and of more perfect elimination of colouring matter, etc. The final chloroform solutions deposited about 80 mgm. of yellow crystalline material from which the toxic oil was easily separated by washing with chloroform. The yellow residue was dissolved in hot absolute alcohol and the solution, to which a drop of hydrochloric acid was added, was placed in the ice-chest. A crop of pale yellow crystals separated and on dilution of the mother liquors further material was obtained. By repeated recrystallisation, two pure fractions were eventually obtained, in greatest quantity a substance "A" crystallising in diamond-shaped pale yellow plates with M.P. 269-271° (Fig. 1) and a small amount of a substance "B", somewhat paler in colour and crystallising in square plates with slightly rounded corners, M.P. 243-4° (Fig. 2). The material A exhibited in a very striking manner the modification of crystalline form caused by traces of impurities. Thus, during the progress of purification, all stages were observed between somatoids, flat boat-shaped crystals, aggregations of plates tapering to a point at the end (see Fig. 3) and the perfectly-formed diamond-shaped crystals of the pure substance.

On account of the properties of these two substances, listed below, it is suspected that they may be flavones.

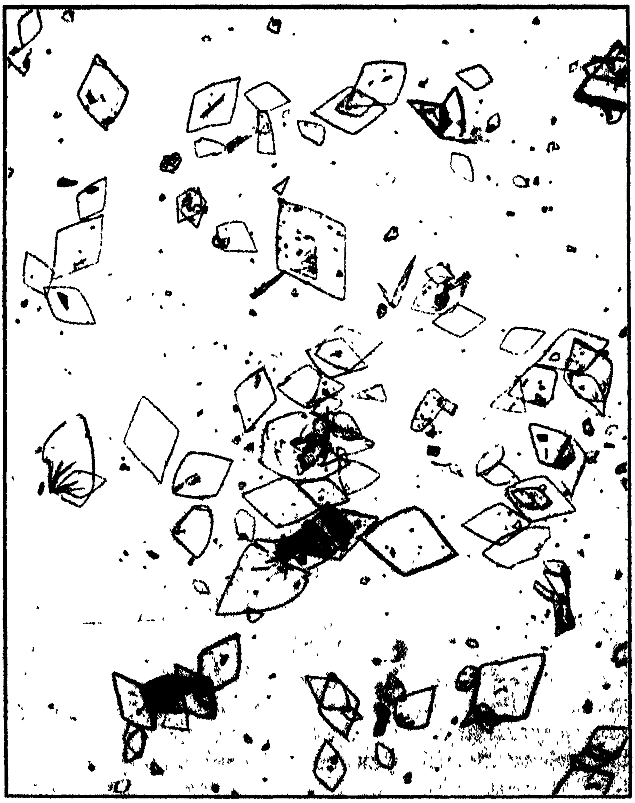


Fig. 1. Substance "A" from *Geigeria aspera*, M.P. 269-71°, ×130.

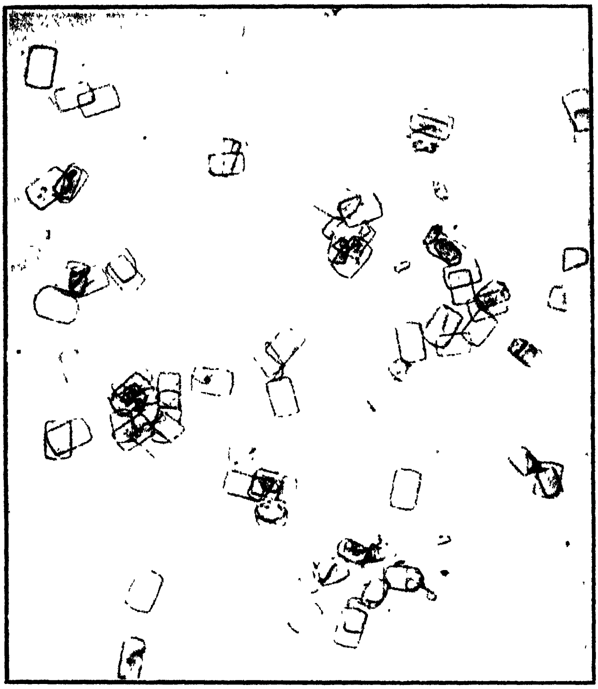


Fig. 2. Substance "B" from *Geigeria aspera*, M P. 243-4°, ×175.

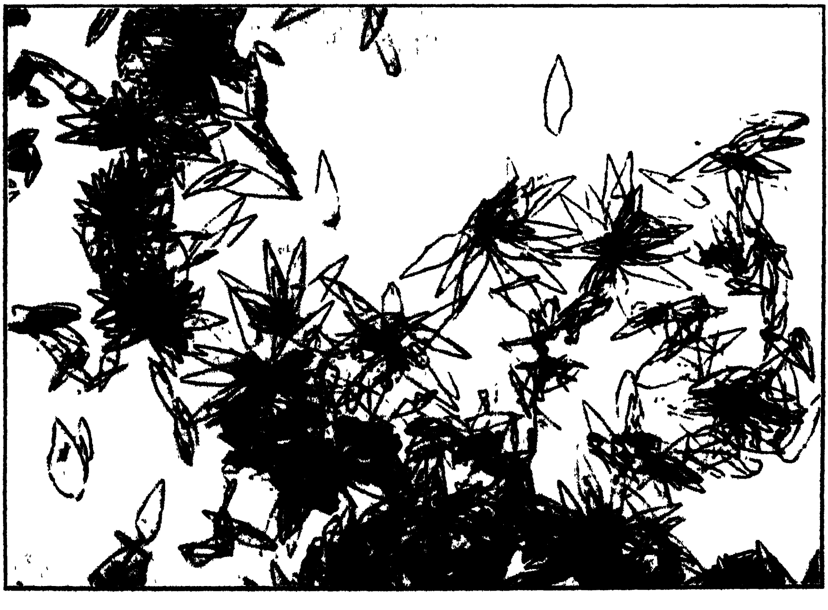


Fig. 3. Somatoids of substance "A" from *Geigeria aspera*, ×85.

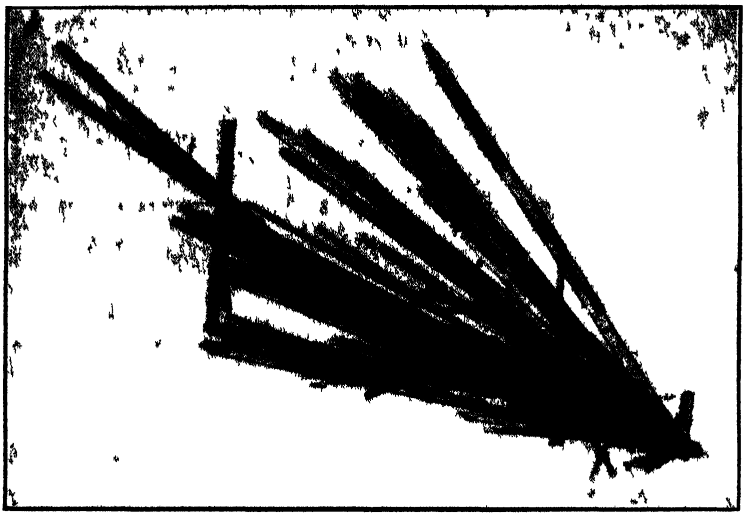


Fig 4 Crystals from solution of substance "A" in concentrated hydrochloric acid, ×80

PROPERTIES OF SUBSTANCES A AND B

A	B
Insoluble in water	Insoluble in water
Insoluble in chloroform (when pure)	Insoluble in chloroform (when pure)
Sparingly soluble in hot alcohol	Sparingly soluble in hot alcohol
Easily soluble in concentrated HCl solution deposits long, bright yellow needles (Fig 4)	Easily soluble in concentrated HCl
Very soluble in dilute Na_2CO_3 solution with bright yellow colour	Very soluble in dilute Na_2CO_3 solution with bright yellow colour
Ferric chloride coloured olive green	Ferric chloride coloured dark olive green
Reactions for ketonic groups negative	Reactions for ketonic groups negative.
Fehling's solution turned dirty green on boiling	Fehling's solution turned dirty green on boiling
Pale yellow diamond shaped plates M P 269 71°	Pale, square plates M P 243 4 .

Analyses indicated the probable formulae $C_{19}H_{18}O_8$ and $C_{19}H_{18}O_7$ for A and B respectively The materials were insoluble in camphor

Micro-analysis *

	C	H	CH_3O
Substance A Found	61·02	4·51	15 36
$C_{19}H_{18}O_8$ requires	60·92	4·86	15·42 (for $2CH_3O$)
Substance B Found	63·58	5·16	
$C_{19}H_{18}O_7$ requires .	63·68	5·03	—

---

* Micro-analyses by Dr O Backeberg, University of the Witwatersrand, to whom we wish to express our thanks

The quantity of B was insufficient to permit of methoxyl determination.

These two materials could also be obtained by dissolving the crude oil in 50 per cent. by volume hydrochloric acid, and setting the solution aside when the yellow compounds slowly crystallised out.

It may be recalled that W. Karrer (1934) recently reported the isolation of a flavone Thapsin from the drug *Digitalis Thapsi*. It had M.P. 224° (uncorr.) and was assigned the formula $C_{15}H_6O_4(OCH_3)_4$ and shown to possess 4 methoxyl and 2 hydroxyl groups. As soon as sufficient material has been accumulated, a further study of these substances present in *Geigeria aspera* will be undertaken.

FINAL PURIFICATION OF THE TOXIC FRACTION.

A phenomenon which proved to be of importance in the final purification of the active material was observed at this stage. Various analyses of final toxic fractions had shown some diversity and it was also observed that preparations which were at first quite fluid gradually lost their mobility on standing in open dishes and that such "old" samples were no longer completely soluble in sodium carbonate solution. Traces of the "A" and "B" materials were also difficult to remove and imparted a dark yellow-brown colour to the preparations.

By dissolving such imperfectly purified material in chloroform and adding, with vigorous stirring, three volumes of petroleum ether, the bulk of the impurities could be removed in the dark resinous precipitate which formed. By a sufficient repetition of the process, preparations of the toxic material could be obtained which no longer gave any colour with ferric chloride solution and were therefore free from the A and B constituents. The increase in viscosity on standing for any considerable period of time still occurred, however, and on occasion the formation was observed of a colourless crystalline substance separating slowly in the bulk of the material (see Fig. 5). The crystals showed marked twinning.

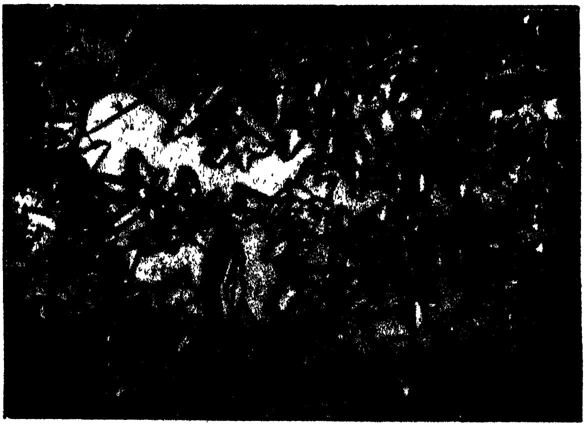


Fig. 5. Crystals of Vermeerin forming in toxic oil (Vermeeric acid) on standing, ×40.

By titurating such preparations with anhydrous ether, added in successive quantities, it was found possible to separate the active acidic oil from the crystals. Where crystallisation had not commenced spontaneously, it could be induced by stirring the oil with dry ether. The crystalline constituent proved to be a neutral principle insoluble in sodium carbonate solution. It could be easily recrystallised from chloroform-petroleum ether mixture and was so obtained in colourless needle-like prisms with M.P. 143° (Fig. 6).

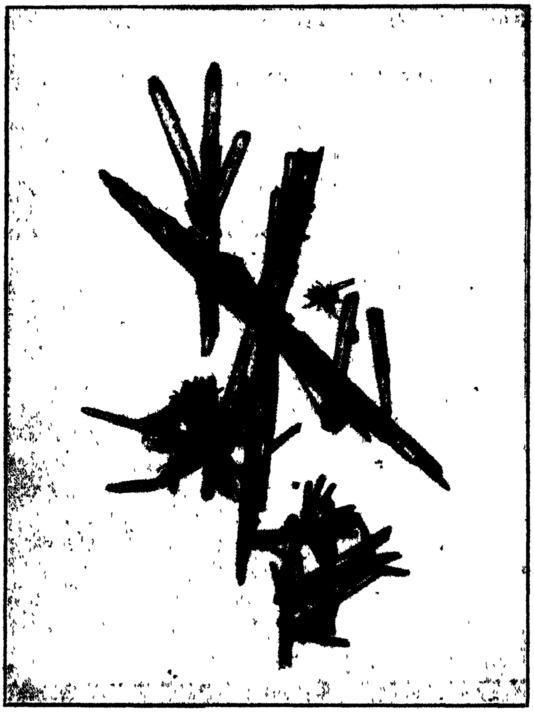


Fig. 6. Vermeerin, the di-lactone of Vermeeric acid, M.P. 143°, ×85.

It was proved that samples of the purified active oil, which originally had been freely and completely soluble in aqueous sodium carbonate solution, slowly but progressively gave rise to this material on standing. Analyses demonstrated that it was formed by spontaneous lactonisation (see below). For analytical purposes, portions of the active oil were dissolved in chloroform and their solutions shaken with aqueous sodium carbonate. The alkaline phase was then repeatedly shaken with fresh portions of chloroform to remove any non-acidic material and the acid transferred to a small volume of chloroform after acidification of the carbonate solution. From this solvent it was precipitated by addition of much anhydrous petroleum ether, centrifuged down, washed with petrol ether and subjected to a short drying (1 hour) in a vacuum desiccator over sulphuric acid. Analyses were made with as little delay as possible and afforded consistent results agreeing with the formula $C_{18}H_{28}O_7$.

Micro-analysis :

	C.	H.
Preparation 1. Found............	60·96	7·96
Preparation 2. Found............	60·90	7·19
$C_{18}H_{28}O_7$ requires................	60·68	7·87

Preparations 1 and 2 were made from different batches of *Geigeria aspera* collected at an interval of about six months. Confirmation of this empirical formula was afforded by analyses of the dilactone as recorded below.

In view of the pharmacological action of this acidic material in causing vermeersiekte or vomiting disease it is proposed to designate it " Vermeeric acid ".

The yield from the batch of material investigated (effective dose for a sheep approximately 2·5 kilos) was 0·5 per cent. The yield of flavones (A and B) was 0·01 per cent.

PROPERTIES OF VERMEERIC ACID.

The material obtained by the process outlined above was a slightly viscous, very pale yellow oil with a pleasantly aromatic smell and devoid of optical activity. It gave the colour reaction with hydrochloric acid which has been previously described and reduced alkaline potassium-permanganate solution in the cold. Vermeeric acid is a dibasic acid as the analysis of its sodium salt and titration experiments demonstrated. It forms a 2:4 dinitrophenylhydrazone and must therefore contain the function $>CO$ or a grouping capable of giving rise to a ketonic group such as an oxygen atom showing keto-enol tautomerism. The reasons for suspecting the presence of such a grouping in Geigerin were fully discussed in the preceding paper.

An amorphous methyl ester was prepared by means of dimethylsulphate. The preparation contained 0·72 per cent. ash.

Micro-analysis :

	C.	H.
Found..........	64·12	7·51
$C_{18}H_{24}O_3\,(CH_3O)_4$ requires..........	64·03	8·73

VERMEERIN, THE DI-LACTONE OF VERMEERIC ACID.

The crystalline material, M.P. 143°, formed from Vermeeric acid on standing (see Figs. 5 and 6) possessed the formula $C_{18}H_{24}O_5$ and was found to neutralise two equivalents of alkali after opening of the lactone rings. It is laevorotatory but the free acid, as was to be expected, proved to be completely inactive. Tests with Brady's reagent revealed the fact that only a very slight precipitate is formed on adding the reagent and this not immediately. From such behaviour it would appear highly probable that the closure of one or other lactone ring involves the structure in Vermeeric acid which, as postulated, is capable of giving rise to a ketonic function in the presence of hydrochloric acid and a ketone reagent. Optical activity must be associated in some way with the closure of the lactone rings.

Micro-analysis:

	C.	H.
Found	67·77	7·45
$C_{18}H_{24}O_5$ requires	67·47	7·56

50·5 mgm. of the substance dissolved in 15 c.c. of absolute alcohol had a rotation of − 0·34°.

$$\therefore \quad \left[\alpha\right]_D^{28} = \frac{-0{\cdot}34 \times 15 \times 100}{2 \times 5{\cdot}05} = -50{\cdot}51^\circ.$$

To this solution was added 5 c.c. of alcoholic potassium hydroxide (approximately 0·08 N solution) and the mixture left at 37° overnight. It was then back titrated after the addition of phenolphthalein.

5 c.c. KOH = 3·42 c.c. of 0·1086 N HCl
Acid back = 0·35 c.c.
∴ Neutralised 3·07 c.c.
= 3·33 c.c. of 0·1 N
∴ Equivalent weight = 151·7

The empirical formula $C_{18}H_{24}O_5$ for 2 lactone groups requires 160.

To the residual solution, the full equivalent of acid was added and the mixture examined in the polarimeter. It proved to be completely inactive.

Like Geigerin and Vermeeric acid, Vermeerin gives the colour test with hydrochloric acid, in which it is fairly readily soluble. The colour was much less intense and somewhat browner than that given by Geigerin but a distinct absorption band was visible with its centre at 546·5 mμ. Two other bands were faintly discernible as in the case of Geigerin and Vermeeric acid. On dilution of the acid, the bulk of the dissolved substance separated in pure crystalline form M.P. 143°. This might indicate that the action of hydrochloric acid, presumably causing some isomeric rearrangement within the molecule is much less marked upon Vermeerin than it is with the other two compounds, a difference possibly to be correlated with the absence of a ketonic functioning group (compare the formulae on p. 519).

During the course of the separation of crystalline Vermeerin from active fractions which had stood some length of time, a certain quantity of another substance was encountered. This was an amorphous solid, devoid of acidic properties, but neutralising alcoholic potash on standing at 37° for 24 hours. 48·8 mgm. left in 5 c.c. alc. KOH (= 3·90 c.c. HCl) required for back titration 1·85 c.c. 0·09709 N HCl.

Neutralised 1·80 c.c. N/10.

∴ Equivalent = 271·6.

The resultant solution was optically inactive.

Micro-analysis:

	C.	H.	M.Wt.	
Found....	61·56	7·89	1028	(by Rast's method).
$C_{54}H_{82}O_{20}$ requires...	61·67	7·87	1050·7	

This substance is characterised by its very sparing solubility in all organic solvents. It has not yet been closely studied but it may be here pointed out that the molecular weight is 4 times the equivalent weight, indicating that there are in all probability 4 lactone groupings in the molecule. The molecular formula $C_{54}H_{82}O_{20}$ corresponds to 3 times the molecular formula of Vermeeric acid less 1 molecule of water thus, $3 \times C_{18}H_{28}O_7 - H_2O = C_{54}H_{82}O_{20}$. Vermeeric acid contains two carboxyl groups per molecule and it may be suggested that possibly some sort of polymerisation involving anhydride formation may have taken place.

VERMEERIC ACID 2:4 DINITROPHENYLHYDRAZONE.

This derivative, which established the presence in the molecule of Vermeeric acid of a ketonic group (or formation of such a group in the presence of acid) was prepared in the following manner:—

About 50 mgm. of Vermeeric acid was dissolved in aqueous alcohol of strength just sufficient to afford a clear solution and after addition of sufficient hydrochloric acid to bring to 2 N, the requisite quantity of hot Brady's reagent was added. The separation of an orange-red dinitrophenylhydrazone commenced rapidly but the precipitate was apt to be sticky. It was washed well in the usual way and dissolved in 1 per cent. aqueous sodium carbonate solution. To the filtered, dark reddish-brown solution was added an excess of hydrochloric acid and the precipitated derivative was centrifuged down and washed. It crystallised from hot dilute alcohol in reddish-orange prisms with M.P. 108–110°.

Micro-analysis:

	C.	H.	N.	
Found. Preparation 1...	54·80	5·94	11·42	(trace of ash).
,, ,, 2...	54·96	5·71	12·52	
$C_{24}H_{34}O_{11}N_4$ requires.....	54·75	5·73	12·17	

The analysis of this derivative occasioned some surprise. The formula expected from the interaction of 1 molecule of Vermeeric acid with 1 molecule of dinitrophenylhydrazine with the elimination of 1 molecule of water would be $C_{24}H_{36}O_{12}N_4$ whereas the substance actually formed appears to contain H_2O less. Considering the ease with which

Vermeeric acid lactonises on standing, it seemed highly probable that the extra molecule of water eliminated owed its origin to closure of one of the lactone rings. That this was indeed the case was proved by microtitration of a specimen of the 2:4 dinitrophenylhydrazone following the method of Clift and Cook (1932).

7·8 mgm. dissolved in 5 c.c. of N/100 sodium hydroxide required for back titration 3·60 c.c. of N/100 hydrochloric acid,

∴ alkali neutralised = 1·40 c.c.

Assuming the presence of 1 carboxyl group, $C_{24}H_{34}O_{11}N_4$ requires ... ... 1·39 c.c.

As already pointed out, there is considerable experimental support for considering the ketonic function in Vermeeric acid to arise through keto-enol tautomerism involving an oxygen atom which in its enol form is capable of lactonising with a carboxyl group. It is thus understandable that during the preparation of the 2:4 dinitrophenylhydrazone of Vermeeric acid, only one lactone ring closes whilst one oxygen atom reacts to form the desired derivative. The 2:4 dinitrophenylhydrazone is readily soluble in sodium carbonate solution, which demonstrates the presence of an acidic function in the molecule.

OXIDATIVE DEGRADATION OF VERMEERIC ACID.

0·5 gm. of the toxic acidic oil was dissolved in 5 per cent. sodium carbonate solution, and to this solution, whilst boiling, was added gradually 40 c.c. of 0·25 N potassium permanganate. On filtration and distillation of the alkaline reaction mixture, a distillate was obtained which gave only the faintest trace of turbidity with Brady's reagent.

The residual alkaline solution was then extracted thoroughly with ether which removed a substance remaining behind as a colourless oil when the solvent evaporated. This material afforded an immediate precipitate of a 2:4 dinitrophenylhydrazone with Brady's reagent. The derivative was washed well and crystallised from hot dilute alcohol in yellow needles, M.P. 160°. It proved to be identical with acetaldehyde 2:4 dinitrophenylhydrazone.

Micro-analysis:

	C.	H.	N.
Found...........	42·99	3·73	24·40
$C_8H_8O_4N_4$ requires..........	42·86	3·60	25·00

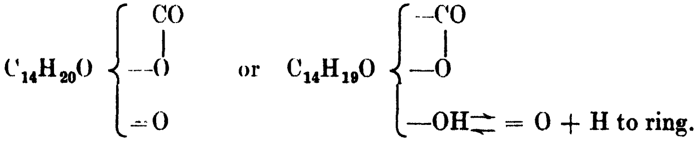
Mixed with authentic acetaldehyde 2:4 dinitrophenylhydrazone of M.P. 159–161° it melted without depression at 159–161°. The yield was 0·23 gm.

After making acid by ether, the residual main solution was again extracted and yielded to ether a small quantity of an acid which was eventually obtained from acetone in aggregates of needles, M.P. 229–30° but the quantity was insufficient for analysis.

These results are purely preliminary but serve to demonstrate the close similarity between Vermeeric acid and Geigerin. Further work upon the constitution of these substances will be undertaken at a later date.

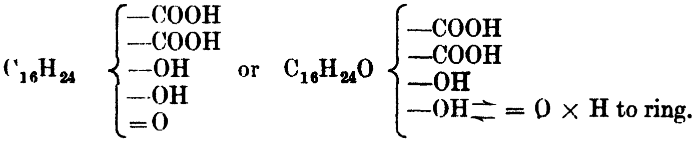
Summarising the chemical information so far obtained, one may represent the three substances Geigerin, Vermeeric acid and Vermeerin as follows, an indication being given of the possible involvement of one atom of oxygen in a keto-enol tautomerism.

Geigerin, $C_{15}H_{20}O_4$.



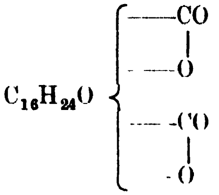
$$C_{14}H_{20}O\begin{cases} CO \\ | \\ -O \\ =O \end{cases} \text{ or } C_{14}H_{19}O\begin{cases} -CO \\ | \\ -O \\ -OH \rightleftharpoons =O + H \text{ to ring.} \end{cases}$$

Vermeeric acid, $C_{18}H_{28}O_7$.



$$C_{16}H_{24}\begin{cases} -COOH \\ -COOH \\ -OH \\ -OH \\ =O \end{cases} \text{ or } C_{16}H_{24}O\begin{cases} -COOH \\ -COOH \\ -OH \\ -OH \rightleftharpoons =O \times H \text{ to ring.} \end{cases}$$

Vermeerin, $C_{18}H_{24}O_5$.



$$C_{16}H_{24}O\begin{cases} -CO \\ | \\ -O \\ -CO \\ | \\ -O \end{cases}$$

SUMMARY.

1. The toxic principle of the Vermeerbos, *Geigeria aspera*, has been isolated. It is a dibasic acid $C_{18}H_{28}O_7$ and has been named "Vermeeric acid". On standing in the air, Vermeeric acid gradually loses two molecules of water forming the crystalline dilactone "Vermeerin" $C_{18}H_{24}O_5$.

2. Vermeerin has M.P. 143° and $\left[\alpha\right]_{D}^{28} = -50{\cdot}51°$ but Vermeeric acid is optically inactive.

3. Both substances, like Geigerin, give a colour reaction with hydrochloric acid but the colour is browner, and in the case of Vermeerin it is of very slight intensity. An absorption band at 546·5 mμ could be distinguished and two other bands were faintly discernible.

4. Vermeeric acid forms a 2:4 dinitrophenylhydrazone soluble in sodium carbonate and reprecipitated by acids. This derivative appears to contain H_2O less than that expected upon the assumption of a simple reaction and it is thought probable that closure of one lactone ring simultaneously occurs. Vermeerin when treated with hot Brady's reagent reacts to such a very slight extent that the absence of any ketonic or keto-enolic function is infered in the undecomposed substance.

5. Vermeeric acid decolorises potassium permanganate in the cold. By oxidation with this reagent in alkaline solution at the boiling temperature there was obtained an acid crystallising in small prismatic needles M.P. 229–30° and a substance which when treated with cold dilute acid immediately liberated acetaldehyde, the 2:4 dinitrophenylhydrazone of which was prepared for identification.

6. Accompanying Vermeeric acid in the plant were found two flavone-like substances, the one with M.P. 269–71° being, in all probability $C_{17}H_{12}O_6(CH_3O)_2$, the other, M.P. 243–4°, having the formula $C_{19}H_{18}O_7$ (methoxyl not determined).

7. Vermeeric acid drenched to sheep in doses of 10 to 15 gm. causes death from acute vermeersiekte within 6 to 48 hours.

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Section IV.

Mineral Metabolism and Deficiency.

MALAN, A. I., DU TOIT, P. J., AND GROENEWALD, J. W.	Studies in mineral metabolism XXXV. The rôle of iodine in the nutrition of sheep	523

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Studies in Mineral Metabolism XXXV.

The Role of Iodine in the Nutrition of Sheep.

By A. I. MALAN, P. J. DU TOIT, and J. W. GROENEWALD,
Section of Biochemistry, Onderstepoort.

INTRODUCTION.

WORK on the feeding of KI to sheep was begun at this Institute in 1929, mainly with the object of collecting information on the advisability of incorporating iodine in licks—a custom which was then being practised on a fairly extensive scale both in this country and abroad by stockmen. The results of the first investigation (1932) suggested:—

(1) That the administration of ·02 grm. KI daily to sheep affected their weight increase adversely when the diet contained insufficient phosphorus.

(2) That on an adequate ration the iodine supplement was without effect on body weight.

(3) That reproduction was apparently interfered with and became abnormal in regard to both the gestation period and the health and vitality of the offspring, when iodine was given, irrespective of the presence or absence of sufficient phosphorus in the diet. These results might obviously have been influenced by the quantity of potassium iodide given and hence in a subsequent experiment (1935) ·002 grm. KI per head per day was given to a group of sheep, ·02 grm. to a second group and ·06 grm. to a third, while complete records were kept of the oestrous cycle of all the animals. The phosphorus intake was adequate and the same in all the groups. It was further realised that the results of the first investigation were probably complicated by the low vitamin A content of the diet which was rectified in the

subsequent work. The results of this experiment showed that oestrum was normal in all the groups and that the KI was without effect on the bodyweight of the sheep. In regard to reproduction, however, the results, although by no means as unsatisfactory as in the first investigation, left much to be desired and suggested that the protein of the basal ration of maize and hay was apparently either inadequate or too poor in quality for normal reproduction and lactation. Hence the present experiment was planned to determine the effect of iodine in the ration of sheep when only poor quality protein was available and secondly when in addition the supply of vitamin A was low or inadequate as might and often does occur under conditions of practical farming in this country.

DETAILS OF EXPERIMENT.

The remaining 28 ewes used in the previous experiment on iodine feeding mentioned above were re-divided into four groups each consisting of seven approximately even and uniform animals. The number of animals in each group was increased to 10 with ewes of the same age and conformation as those already available.

The four groups of 10 sheep were placed on the following rations:—

Groups.	*Rations.*
1	Hay *ad lib*, 225 grm samp and ·05 grm KI,
2	Ration of group 1 plus 200 grm green feed.
3	Ration of group 2 plus 50 grm blood meal.
4	Ration of group 3 but omitting the KI

10 grm. bonemeal were added daily to the ration of each sheep.

The management and individual feeding of the sheep were essentially the same as in the earlier work of which the details were given in the first publication (1934). The samp (maize endosperm)was almost always completely consumed and this ensured the regular ingestion of the KI which was added in solution to the maize after the latter had been weighed and placed in the feeding boxes. The green feed in groups 1, 2 and 3 was given in separate feeding boxes in the individual feeding pens in which all the sheep were placed daily at 2 p.m. and left until 8.30 a.m. the following morning. Hay was available in racks in the common run to which the sheep had free access when they were not in the feeding boxes. Water was always available in the common gravelled paddock. The experiment was continued long enough for full observations on one period of reproduction and lactation; it was started in February, 1935, and concluded at the end of February, 1936.

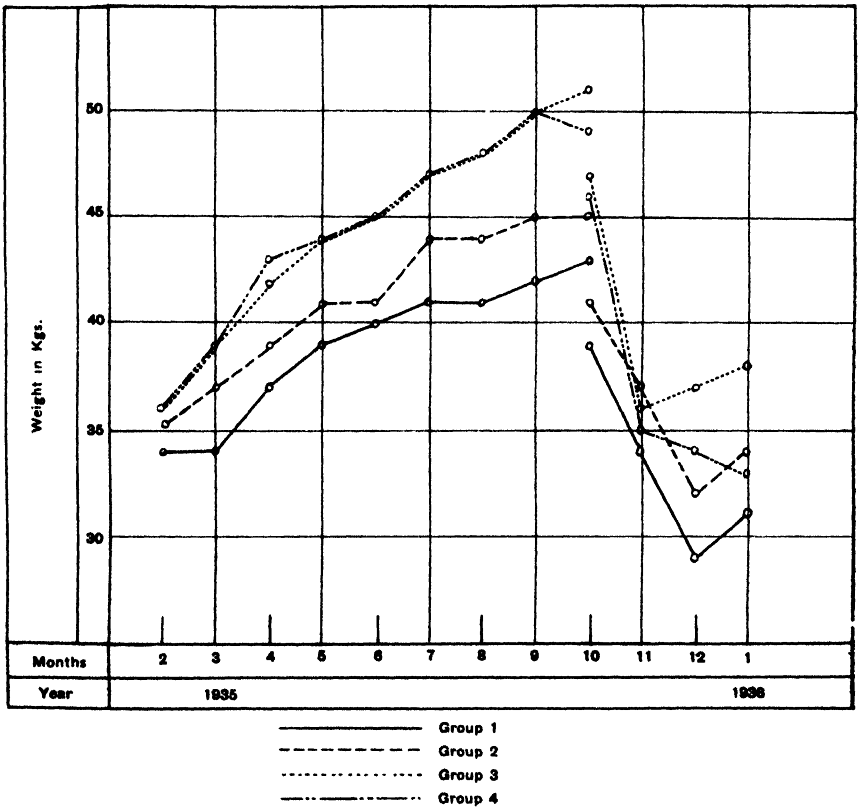
As in the previous work observations were made on weight increase, food consumption, wool production and reproduction. In view of the regularity of oestrum, previously observed, further observations were not made in regard to this matter but the ewes were served in May, 1935, when all except one became pregnant.

RESULTS.

(1) *Bodyweight.*

The sheep were weighed at monthly intervals and the average weights per group are given in figure 1 below. The weight of the non-pregnant ewe in group 4 is not included in the curve for that group.

FIGURE 1.



In order to compare the effects of lambing on bodyweight in the different groups the weights of all the sheep just prior to lambing were averaged for the separate groups and the averages plotted on the same ordinate. By this means the curves are more directly comparable as the gestation periods of all the sheep but one, which was not pregnant, and the lactation periods of those that reared lambs are made to synchronize. The effect of shearing is indicated by a perpendicular drop in the curves of October, 1935.

A glance at Figure 1 indicates that groups 3 and 4 receiving a protein supplement of 50 grms. bloodmeal daily differed significantly from the other two groups in weight. As no difference in bodyweight existed between these two groups prior to lambing, however, their more rapid increase in weight cannot be associated with the KI supplement which only one of the two groups was receiving but with the greater nutritive value of the blood meal diet. Group 4 was apparently more adversely affected by lambing and lactation than group 3 but this is readily explained by the fact that the former group reared 8 lambs and the latter only 5, which would naturally favour weight increase in group 3 subsequent to lambing. When groups 1 and 2 are compared it would seem that the presence of greenfeed in the latter group had no noticeable effect on weight increase at any stage of the experiment. When the post parturition periods of all four groups are compared it is seen that the weights of the animals in group 4, which reared the largest number of lambs, continued to drop, while the others showed an earlier recovery.

(2) *Weight of Lambs.*

Weekly body weights of the lambs were taken as these might serve to indicate the milk production of the ewes. The weights are given in Table 1 below:—

Group I.

TABLE 1.

No. of Ewe.	Gestation Period. Days.	Sex of Lamb.	Weights of Lambs.				Remarks.
			Birth Wt.	14th Day.	21st Day.	50th Day.	
			lb.	lb.	lb.	lb.	
32900.	153	Ewe	7	—	—	—	Died two days after birth.
41795.	154	Ram	6	—	—	—	Died one day after birth
32917.	149	Twin Ewes	5 3·5	— —	— —	— —	Died one day after birth.
32916.	149	Ewe	6	9·5	10·5	15	—
41825.	147	Ram	5·8	—	—	—	Died one day after birth.
32888.	151	Ewe	7·5	—	—	—	Died one day after birth.
41787.	150	Ewe	7	15	17	22·5	—
32885.	149	Ram	6	—	—	—	Died three days after birth.
32905.	146	Ram	5	—	—	—	Died three days after birth.
32889.	—	—	—	—	—	—	Ewe died at beginning of experiment.
TOTAL			38·8	24·5	27·5	37·5	
AVERAGE			5·9	12·3	13·8	18·7	

Group II

No. of Ewe.	Gestation Period. Days.	Sex of Lamb.	Weights of Lambs.				Remarks.
			Birth Wt.	14th Day.	21st Day.	50th Day.	
			lb.	lb.	lb.	lb.	
32887.	149	Ram	6·5	9·5	11	16	—
32902.	150	Ram	6·5	16·5	16·5	22·5	—
41693.	151	Ewe	5·5	—	—	—	Died one day after birth.
41868.	147	Ram	6·5	—	—	—	Died two days after birth.
32911.	151	Ram	9	—	—	—	Born dead.
32896.	151	Ewe	7·5	13·5	15	22	—
31817.	151	Ewe	6·8	13·5	15	19	—
32893.	153	Ewe	7	13	15·5	22	—
32891.	152	Ram	7·5	9·8	10·5	15·0	—
32906.	—	Ewe	7·2	15·5	16·5	21·5	—
	Total		70	91·3	100·0	138·0	
	Average		7	13·0	14·3	19·7	

Group III

No. of Ewe.	Gestation Period. Days.	Sex of Lamb.	Birth Wt.	14th Day.	21st Day.	50th Day.	Remarks.
32904.	148	Ram	7·5	17·0	19·0	22·5	—
32895.	150	Ram	8·4	—	—	—	Died one day after birth.
32907.	153	Ewe	7·0	—	—	—	Died two days after birth.
41698.	150	—	8·5	—	—	—	Born dead.
41789.	152	Ewe	8·5	15·0	18·5	235·	—
41881.	167	Ewe	6·5	8·0	8·5	—	—
32903.	150	Ram	7·5	15·0	17·0	23·5	—
32894.	149	Ram	8·0	13·0	14·5	18·0	—
32894.	149	Ram	8·0	13·0	14·5	18·0	—
32908.	156	Ram	8·5	—	—	—	Born dead.
	Total		70·4	68·0	77·5	85·5	
	Average		7·8	13·6	15·5	21·8	

Group IV

No. of Ewe.	Gestation Period. Days.	Sex of Lamb.	Birth Wt.	14th Day.	21st Day.	50th Day.	Remarks.
32886.	154	Ewe	7·5	22·0	22·5	27·5	—
32898.	153	Ram	6·5	15·5	17·5	19·5	—
32883.	—	—	—	—	—	—	Ewe died at beginning of experiment.
42020.	151	Twin Rams	5·0	—	—	—	Died one day after birth.
			5·5	10·5	13·5	16·0	
32901.	128	Ewe	6·5	13·0	15·0	21·0	—
41798.	—	—	—	—	—	—	Not pregnant.
32910.	152	Ewe	7·0	15·0	16·5	23·0	—
32914.	150	Ewe	7·5	10·0	13·0	21·0	—
41754.	—	—	—	17·0	20·0	28·0	
32897.	155	Ram	8·5	10·0	13·0	21·0	—
	Total		54·0	113·0	131·0	177·0	
	Average		6·7	14·1	16·4	22·1	

The number of lambs reared in this experiment is too small to justify conclusions being drawn; in group 1 for instance only two lambs remained alive. One point is remarkable, however, viz. that in group 4, where 8 lambs were reared, all were strong and showed an increase which is perhaps just suggestive of better feeding conditions in this group than in group 2, for instance, where at least two lambs were below normal weight seven weeks after birth. Although milk production is admittedly important the available information, due mainly to the comparatively large number of lambs that did not survive the lactation period, is insufficient to justify definite conclusions in regard to the milk yield of the ewes as judged by the weight increase of the lambs.

Food Consumption.

The food consumption of the individual animals was recorded by weighing back all the feed left over in the feeding boxes at weekly intervals. From the following figures which represent the percentages of food eaten per group for the full period it is obvious that no significant difference exists between the groups.

Groups.	*Maize Eaten.*	*Green Feed Eaten.*
1	86 per cent.	No green feed given.
2	86 per cent.	98·3 per cent.
3	90 per cent.	99 per cent.
4	92 per cent.	98 per cent.

The absence of significant differences in the quantities of maize and green feed consumed by the respective groups indicates that groups 3 and 4 were daily consuming the additional protein contained in the 50 grm. of blood meal given, over and above that contained in the rest of the ration. Although this additional protein effected a more rapid increase in weight during pregnancy it was apparently without effect on the weight during lactation, during the first month of which all the groups showed remarkable decreases in weight. Hay was consumed at the rate of approximately 1·5 lb. per head per day.

Wool Production.

The weights of wool produced are given in Table 2.

A glance at Table 2 reveals that groups 3 and 4 receiving the extra protein in their ration produced significantly more wool per head than groups 1 or 2 on maize and hay. This result confirms the observation made in regard to body weight that half a pound of maize, and hay of the quality usually available in this country, even if given ad lib, do not supply sufficient protein for wool production and reproduction.

TABLE 2.

Weights of Wool in Kgs.

	Group 1.		Group 2.		Group 3.		Group 4.	
	Nos.	Wt. of Wool.	Nos.	Wt. of Wool.	Nos.	Wt. of Wool.	Nos.	Wt. of Wool.
	32900....	3·6	32887...	3·8	32904...	4·1	32886...	4·3
	41795....	2·9	32902...	2·7	32895...	3·6	32898...	5·0
	32917....	♌	41693...	3·2	32·07...	3·8	32883...	♌
	32916....	3·4	41868...	3·6	41698...	4·3	42020...	3·6
	41825....	3·2	32911...	4·3	41789...	3·6	32901...	4·6
	32888....	4·1	32896...	4·1	41881...	5·5	41798...	5·0
	41787....	3·2	41817...	3·4	32903...	5·0	32910...	4·3
	32885....	4·3	32893...	4·1	32894...	4·5	32914...	5·0
	32905....	♌	32891...	3·6	32908...	4·3	41754...	3·4
	32889....	♌	32906...	—	—	—	32897...	4·8
TOTALS........		24·7		36·4		34·6		40·0
AVERAGES.....		3·5		3·6		4·3		4·4

It must be pointed out, however, that the weights of the grease wool are given in Table 2 and that the increased wool production in groups 3 and 4 must be regarded as tentative until further studies on the fleeces have been completed.

The KI supplement given to all the groups except 4 was clearly without effect on the weights of the wool produced.

Reproduction.

Details in regard to reproduction are given in Table 1.

Table 1 reveals several interesting points: With the exception of group 4 where no KI was given reproduction was very unsatisfactory. In group I where greenfeed was omitted and vitamin A deficient only 2 lambs of the nine born survived. In groups 2 and 3 receiving greenfeed and greenfeed plus bloodmeal respectively reproduction was not normal. In group 4 where 8 ewes lambed, 9 lambs were born, while one of the twin lambs died 3 days after birth. It is remarkable that reproduction was poor only in the three groups receiving supplements of KI irrespective of other factors such as for instance absence of greenfeed in group 1, or supplementary protein feeding in group 3. The results have been summarized as follows:—

Groups.	Remarks on Treatment.	Remarks on Production.
1..........	Hay, maize and KI given. Vitamin A very low; protein poor in quality	All the ewes lambed; 7 lambs died within 3 days and 2 lambs reached the age of 7 weeks when they were discharged.
2..........	Hay, maize, greenfeed and KI. Vitamin A adequate but protein still poor in quality	All the ewes lambed; 3 lambs died within 3 days and 7 reached age of 7 weeks (discharged).
3..........	Hay, maize, greenfeed, bloodmeal and KI; quantity and quality of protein improved	All the ewes lambed; 4 lambs died within 3 days and 5 were discharged at 7 weeks.
4..........	Hay, maize, greenfeed and bloodmeal. The ration is the same as that of Group 3, except that KI was omitted	One ewe not pregnant and one died in the course of the experiment. All the other ewes lambed. Of the 9 lambs born (1 pair of twins), 1 of the twins died, 3 days after birth. All the other were discharged when 7 weeks old.

Discussion.

The bodyweights and data on food consumption can justifiably be dismissed without further discussion as the difference in bodyweights between groups 3 and 4 on the one hand and 1 and 2 on the other was not associated with the KI supplements, as already stated.

In regard to reproduction, however, the best group, as judged by the number of lambs reared was the one which did not receive KI in its ration. In the poorest group, viz. group 1, the vitamin A intake was too low for normal reproduction (Malan et al 1932) and it would again appear as in the earlier work just quoted that the vitamin A deficiency intensified the deleterious effect on reproduction of the KI in the ration. It is clear, however, from the results obtained in group 2, as was also concluded from the 1935 experiment, that the supply of 200 grm. greenfeed per animal per day, which provided adequate vitamin A, as reference to the vitamin A content of the liver will verify, improved but did not remove the cause of poor reproduction. The addition of bloodmeal to the ration, as was done in group 3, brought about no further improvement until the KI was omitted from the ration (group 4), when reproduction was significantly benefitted. Although the ration of group 4 contained bloodmeal it could probably easily be improved upon both in quality and palatability. Still, reproduction in this group was very satisfactory in spite of the one lamb of twins dying a few days after birth. On the addition of KI to this ration, however (group 3), reproduction was decidedly abnormal with 4 lambs living less than 3 days. As a matter of fact the effect of the bloodmel supplement seems to have been entirely masked by the deleterious effects of the KI supplement in group 3.

In the absence of an ideal ration it is impossible to state from a consideration of the available data that ·05 grm. KI administered daily to sheep will produce deleterious effects on reproduction under all conditions, but there can be no doubt that the only common factor in all three groups, showing unsatisfactory reproduction was the KI supplement, which on being omitted improved reproduction very considerably and significantly. The number of animals in the different groups is small for an experiment of this nature where many incidental factors might influence the rearing of lambs, especially when deleterious factors have been introduced. No final conclusion can be drawn in regard to the effect of KI on milk production.

Vitamin A Content of Livers.

The vitamin A content of the livers of some of the lambs that died, was determined according to the method of Rosenthal & Erdelyi (1934) with remarkable results. The livers of four lambs in group 1 showed no vitamin A or only slight traces.

$\frac{N}{100}$ $KMnO_4$ solution was used as the standard colour against which the colour due to the presence of vitamin A was read in a 50 mm. Leitz colorimeter, the standard being placed at 10 on the millimeter scale. Readings to about 40 on the mm. scale could easily be taken and a trace is intended to mean a slight coloration but too ill-defined to be compared with the $KMnO_4$ solution.

It should be remembered that the quantity of vitamin A present is inversely proportional to the colorimetric reading; a trace indicates a reading greater than 40 against the Standard at 10 while stronger solutions can be expressed in terms of their colorimetric readings. 75 grm. of liver were used for each determination and the comparative quantities of vitamin A were then calculated for the total weight of liver as follows:—

$$\frac{(\text{reading of standard}}{(\text{reading of unknown}} \times \frac{\text{total weight of liver})}{75 \qquad)}$$

The values so obtained are obviously now directly proportional to the vitamin A content of the liver.

In groups 3 and 4 where greenfeed was given to the ewes the average values of the livers of the animals killed was approximately 250 while the comparative value of those killed in group 1 was not more than 10, suggesting that the vitamin A of the livers of the sheep in the latter group was only about a twenty-fifth of that of the two former groups. The livers of the lambs of the ewes in the groups mentioned show similar differences, as would be anticipated from the fact that the intake of one group was practically devoid of carotene while the other groups received daily approximately 20 mgm. carotene per head in the greenfeed, as determined by chemical analysis. Vitamin A is apparently supplied by the mother to foetus as was also observed and verified in the case of rats by Dann (1934). The livers of the lambs in group 1 contained little or no vitamin A and the livers of the mothers were practically devoid of it.

SUMMARY AND CONCLUSIONS.

When the daily ration of Merino ewes contained ·05 gm. KI for a period of about twelve months no effects were observed in body-weight and food consumption. Reproduction was, however, abnormal in all the groups receiving the KI supplement, the effects of which were more pronouncedly deleterious in sheep on a carotene low diet than when 200 grm. greenfeed were supplied daily. The response to increased protein feeding in the form of bloodmeal was marked by the detrimental effect of KI on reproduction. As all the rations can easily be improved both in quality and palatability it cannot be inferred that the quantity of KI given will affect reproduction in sheep adversely under ideal feeding conditions. The conclusion is justified, however, that when greenfeed is absent or inadequate as frequently happens in practice good quality protein is not available and the incorporation of KI in sheep licks is distinctly dangerous and may even cause losses due to abnormal reproduction.

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Section V.

Pathology.

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The Occurrence of Congenital Porphyrinuria (Pink Tooth) in Cattle in South Africa (Swaziland).

By P. J. J. FOURIE, Section of Hygiene, Onderstepoort.

INTRODUCTION.

THIS communication deals with a grade short horn herd, in which some 13 cases of what has been diagnosed as congenital porphyrinuria occured after the introduction of a certain short horn bull as sire. The bull himself does not show any symptoms of the condition. In addition to the occurrence of the condition, the symptomatology and haematology of four clinical cases, as well as the pathology of two other cases will be discussed. The nature of the pigments present in the blood, urine, faeces (one case) and various organs (2 cases) will be discussed independently by Rimington elsewhere in this journal from the chemical point of view.

Congenital porphyrinuria is an abnormality in pigment metabolism, which rarely occurs in man and some animals. Günther (1925) devotes an entire chapter to the discussion of the whole condition under the heading " Haematoporphyrie ". The abnormality is very extensively reviewed, particularly as it occurs in man. He points out that haematoporphyrinuria can only be regarded as being present when abnormal quantities of porphyrins are indentified in the urine, as traces occur normally in urine. Even when in various diseases, porphyrins are present in the urine somewhat in excess of the normal amounts, one cannot speak of a haematoporphyrinuria, and their presence in urine and faeces in certain organ diseases, is of no diagnostic significance from a clinical point of view. When abnormal amounts of porphyrins are present, it indicates a constitutional anomaly in pigment metabolism. Such individuals have a tendency towards nervous irritability, sleeplessness and neurosis.

Clinically Günther recognises two forms of the condition: (1) Acute haematoporphyrinuria, which comprises (*a*) the idiopathic form, and (*b*) the toxic form; (2) congenital haematoporphyrinuria.

(1*a*) *Acute idiopathic haematoporphyrinuria.*—Although a hereditary tendency is not well marked in the acute idiopathic cases, the case of Barker and Estes, quoted by Günther is nevertheless significant, as four sisters developed symptoms of the condition at more or less the same age (18 to 22 years). There would seem to be evidence that the mother and grandmother (of these four sisters) on the mothers side, had attacks of vomition, colic, obstipation and discolouration of the urine. Nervous disorders seem to occur in families of affected individuals. The symptoms include: intestinal disturbances—spasm of portions of the small intestine, atony of other portions of gastro-ontestinal canal, with severe constipation; nervous disturbances—sleeplessness, paralysis, etc., markedly increased excretion of what is described as haematoporphyrin in urine and faeces, at the time of the acute attack, but the subsidence of the excretion of these substances during the intervals between attacks; pigmentation of the skin; no well defined changes are regularly present in the blood, photosensitization is absent. Intervals between attacks vary from weeks, months, to one to two years and more. Pigmentation of the bones is not a characteristic of this form of the condition. The cause is not known.

(1*b*) *Acute toxic haematoporphyrinuria.*—These acute toxic cases are differentiated from the idiopathic form in that the use of substances such as sulphonal and trianol bring on the attacks which are usually fatal, ending in ascending paralysis. Günther would seem to suggest that the insomnia which leads to the abuse of these narcotics may indicate a constitutional abnormality in which the use of the drugs mentioned would result in an acute attack of haematoporphyrinuria. The use of sulphonal favours attacks especially in women.

2. *Congenital haematoporphyrinuria.*—Whereas in the acute form 92 per cent. of the reported cases were females, the congenital form seems to occur more frequently in males (78 per cent.). Cases have repeatedly been found in more than one member of the same family and the presence of a hereditary factor is suggested, but not yet definitely established. In one case (also Gray's 1926 case) the parents were related. The symptoms are: (1) Photosensitization, especially during spring, producing lesions of exposed parts of the skin (Hydroa Aestivale), particularly of the ears, nose, cheeks, eyelids, upper lip, fingers, the back of the hand, nails. These lesions may produce very marked disfiguration and deformity. It is remarkable that lesions do not occur on the chin and the mouth region. There is pigmentation of the skin, hypertrichosis, frequently with excessive growth of hair under the arms, eyebrows, beard from the chin and sometimes of the pubic hairs. The hair of the head may be coarse.

(2) The excretion of porphyrins in urine and faeces. The urine is of reddish brown colour. The brownish discolouration is due to the temporary presence of large amounts of urobilin and urofuscin. In one case (Fischer and Schumm) quoted by Günther, the crude porphyrins determined quantitatively were excreted in unchanged amounts for a period of three years. In some cases porphyrins are

present in the serum. Schumm, quoted by Günther, found amongst others, uroporphyrin, whilst Fischer also quoted by Günther, believes that Coproporphyrin is present in the serum. Sweat and saliva are free from porphyrins. The teeth may be yellowish white, the roots reddish brown.

In some cases [Mackey and Garrod (1922) and (1926) and Ashby (1926)] the temporary and permanent teeth are pink. There is dark brown discolouration of the bones whilst tendons and ligaments remain unpigmented. In some cases there is a severe anaemia (anisocytosis, poikilicytosis, polychromasia, punctate basophilia) whilst in others normal counts are present. The course of the disease is influenced by a number of factors. Some individuals reach ages of 50 and 65 years, others die younger from some intercurrent disease, but if sufferers from the condition take certain precautions to protect themselves against harmful rays, the bad effects of the condition can be controlled to a considerable extent. Up to that time no clinical case in an animal was available for examination and study.

Garrod (1892) has shown that haematoporphyrin is frequently present in minute amounts in healthy individuals and in larger amounts in urines of sufferers from a great variety of diseases.

Mackey and Garrod (1922) and (1926) describe a case of congenital porphyrinuria in a boy in whom at 4½ years of age there was delayed ossification of the ulna and some carpal bones, but ossification was normal at 9½ years. There is excessive vulnerability of the skin to slight injuries, for instance the rubbing of the ear with cotton-wool soaked in spirits, produces a bleeding surface. Later on the spleen and the liver become enlarged. The number of red cells remained at a level round about 4·5 millions when counted in 1921, 1925 and 1926. There are no gross changes in the differential counts, but there would seem to be an increase in the number of cells described as endothelials. There is anisocytosis, poikilocytosis, polychromasia, punctate basophilia and normoblasts. In confirmation of the views expressed by Price Jones and Robitschek, quoted by Mackey and Garrod (1926), these changes are interpreted as being indicative of an actively functioning bone marrow. This is regarded as a compensatory reaction to haemolysis which is taking place, but it is not believed that haemoglobin from broken down corpuscles is the parent substance of the porphyrins excreted. The hyperactivity of the bone marrow maintains the red cells at a fairly high level, so that no anaemia develops in spite of haemolysis, which is thought, may be due to the action of light on the sensitized blood in the peripheral circulation. The authors quote Schumm and Fischer who have shown that the staining of the bones is due to uroporphyrin and not to coproporphyrin. The authors claim that at that time their case and that of Ashby (1924), are the only ones amongst the recorded congenital porphyrinuria cases, in which there is conspicuous discolouration of the teeth including the enamel. They are inclined to believe that the explanation for this is that large amounts of porphyrins were available for staining these structures at the time they were being formed even during foetal life.

In the case of Ashby (1924) already referred to there is in addition to the pink teeth, also photosensitization and red urine. These changes were present at birth. A cream containing quinine seems to protect the skin against the harmful rays. Gray (1926) describes a case in a girl, who was apparently healthy up to 5 years of age. In addition to the presence of red urine, skin lesions, yellowish brown teeth, there is considerable hirsuties on the exposed parts of the body. She is one of a family of seven, all of whom are free from the disease, but the parents are first cousins.

Garrod (1923) points out that of the few known cases of congenital porphyrinuria in humans, in several instances more than one member of the same family suffered from the same complaint. This is apparently the only evidence suggestive of the possible hereditary nature of the condition, apart from other factors, such as its greater incidence in males, in which respect it strongly resembles other conditions such as albinism and alcaptonuria which are thought to be transmitted as recessive hereditary characters. Garrod is inclined to agree with Fischer who, at that time, favoured the view that porphyrin is an intermediate product in the conversion of haemoglobin into bilirubin.

Mason, Courville and Ziskind (1933) state that in all 27 cases of congenital porphyrinuria are recorded. They describe four cases of the acute idiopathic type of the disease. The symptoms recorded include, amongst others, abnormal urine, abdominal pain, disturbances of the nervous system, with parenchymatous degeneration of peripheral nerves, ganglion cells of dorsal root and sympathetic ganglia and central nervous system, ending sometimes in death from ascending paralysis.

Borst and Köningsdörffer (1929) give a very exhaustive description of the pathology of the case Petry. Further reference will subsequently be made to their pathological findings and to their views on the pathogenesis of the condition.

Hegler, Fraenkel and Schumm (1913) describe a case of a young woman in whom photosensitization was absent, there was no red urine at the time of the examination, but on post mortem examination the bones were found to be discoloured.

Fraenkel (1923) injected porphyrins prepared from the urine of Petry into experimental animals. The pigment was deposited in growing bone and he was further able to show that after fractures in adult animals, pigment was deposited only in the callus and not in the rest of the skeletal bones. He is of opinion that pigment once laid down in bone, remains permanently. He states that all cases of congenital porphyrinuria in man are not photosensitive and this he also finds in experimental cases.

Fischer, Hilmer, Lindner, & Pützer's (1925) work concerning the porphyrins present in Petry, is adequately reviewed by Rimington elsewhere in this journal.

Turning now to the recorded cases in domesticated animals (bovines and swine), as far as one can make out from the descriptions given, there is not a single instance in which an animal showing clinical symptoms was available for examination. In spite of the fact that Tappeiner (1885) (paper not available) quoted by Poulsen (1910) and Schmey (1913), showed that a pigment which he described as haematoporphyrin, is present in bone of a swine, which had socalled ochronosis, confusion of this condition in animals with true ochronosis of humans was maintained in veterinary literature for a number of years [see the cases of Moselman and Hebrant(1898), Rémy, Brouvier and others quoted by Schmey (1913)].

Mettam (1910) (pig) and Witte (1914) (bull and calf) describe as ochronosis what are almost certainly true cases of porphyria. Witte's cases are of special interest in that the affected bull was the sire of an affected calf, but a calf from the same bull out of a different cow was normal.

Poulsen (1910) was the first author to recognise that ochronosis of animals and man are two entirely different conditions. Whereas in man the pigment in ochronosis is melanin, in the ochronosis as described in animals the pigments are haemosiderin and haematoporphyrin. In man the bones as well as tendons, ligaments and cartilage are pigmented, whereas in animals only the bones are pigmented and tendons, cartilage and ligaments are entirely free from pigment.

Schmey (1913) briefly reviews human cases of ochronosis but gives full details of 15 cases of ochronosis described up to that time in animals. He quotes the spectroscopic examination of the pigment according to various authors: (1) Moselman and Hebrant (1898)—heifer—melanin. [Poulson (1910) states that the chemical reactions described and the spectroscopic measurements recorded by these authors, do not justify them in concluding that they were dealing with the pigment melanin].

(2) Poulson (1910)—case No. 3—cow—haematoporphyrin in bones (haemosiderin in bone marrow).

(3) Tappeiner (1885)—bones of a pig—haematoporphyrin.

(4) Ingier (1911) (2)—pig—not a blood pigment—probably melanin or a derivative of chlorophyll.

(5) His own cases (1912)—2 pigs—haemoglobin derivative, probably that of acid haematin, but not haematoporphyrin. (The two bands which he describes may equally well be that of acid uroporphyrin as that of acid haematin).

Schmey further quotes Schenk and Colberg each of whom describes discolouration of the enamel of the teeth in a cow and a three day old calf respectively.

Schmey emphasises that ochronosis in humans is an entirely different condition to what has been described as ochronosis in animals and that osteohämatochromatosis more suitably describes the condition in animals. His lead in thus designating the anomaly in animals was subsequently followed by Teutschlaender (1914); Maraev (1928) and Cohrs (1931) (Osteohämochromatose); Kitt (1921) (Haemochromatosis ossium); and by Joest (1926) (Hämochromatose).

Teutschlaender (1914) states that at that time 19 cases (including his own) of socalled pseudo-ochronosis, in animals are reported in the literature. In one of his own cases as well as one of Poulsen's referred to by him [also a case of Fikentscher (1930)], only a proportion of the bones were pigmented. In some cases the pigment is not uniformly present throughout the bones, but is laid down in alternating darker and more lightly strained rings.

Fikentscher (1930) describes changes in organs and bones from a bovine, concerning which nothing clinically abnormal was known. By the use of a fluorescence microscope the presence of porphyrins could be demonstrated. His work was confirmed by the chemical observations of Fink quoted by him.

OWN CASES.

History.

In view of the fact that these cases are the first to be described clinically, it is proposed to give a detailed description of the history of the whole herd. Mr. Cassie, Anniswells, Bremersdorp, Swaziland, introduced a pure bred short horn bull No. 7015, to his grade short horn herd in 1931. The owner has for some time been grading up his herd with short horn bulls. Three bulls were used. The first bull is not in any way related to bull 7015, but the second bull is out of the same herd, as the sire of bull 7015. After the introduction of bull 7015 he found that some of his young stock began to do badly, soon after weaning. Such animals loose condition and develop a rough staring coat. In some cases scabs are present in the middle of the back, where the hair parts; crusts and scabs may be present around the eyes and nose and there may be a nasal discharge. The urine is red and may remain so for months on end. These cases do not respond to treatment for redwater (piroplasmosis) by injection of trypan blue. In December 1934 one such animal was killed and apart from the red urine and a certain amount of discolouration of internal organs, Mr. Cassie did not observe anything abnormal, and the carcase was given to the natives for food. When the natives cut up the carcase they saw the pink teeth and when Mr. Cassie's attention was directed to this he noticed that not only the teeth, but all the bones were of a reddish brown colour. One of the natives who consumed a large amount of this meat had violent colic, but none of the others reported sick. During 1935, whilst on holiday in Swaziland, the Principal Veterinary Officer, Mr. W. A. Elder, M.R.C.V.S., consulted me concerning these cases. On examining the mouths of these animals, I was amazed to see all the teeth (temporary and permanent) of a brownish pink colour. Not having seen anything like it before, I could at the time only speculate as to the nature of the condition and ventured rather boldly an opinion of a possible haemopoietic disturbance. However, arrangements were made for the collection of specimens and these were subsequently submitted by Elder, when the condition was immediately recognised to be osteochaemochromatosis as described by Kitt, Joest and Cohrs, already referred to. A piece of bone was handed to Rimington in order to determine the nature of the pigment present. After obtaining the pigment in solution, he was able to indentify it as porphyrin spectroscopically and chemically and the diagnosis of porphyrinuria was definitely established.

During 1935 Cassie had more or less 270 head of cattle. Of these 67 were cows (it is not possible to give definite figures of the numbers of breeding stock during 1931, 1932, 1933 and 1934). Since the first case was observed in December, 1934, and the beginning of 1936, 12 cases of the condition were found in Cassie's herd (porphyrins were determined chemically and spectroscopically in six of these 12 cases) and one case was found on an adjoining farm A (see below). The bull 7015 was occasionally used on this farm A and this one case is his offspring. On the adjoining farm C on the South side, the bull was never used as a sire and no cases of the condition were observed in any of the animals there. Bull 7015 was the only bull used in Cassie's herd at that time.

E.

	Adjoining farm 260 head of cattle	Cassie's farm 270 head of cattle, 67 cows, 1935	Adjoining farm South side, bull 7015 not used.	
N.	A.	B.	C.	S.
	1 case of porphyria sired by bull 7015	12 cases of porphyria all sired by bull 7015	No cases of porphyria observed.	

W.

Of the 13 cases, 10 are males and 3 are females. In 3 cases the abnormality was seen by Cassie immediately after birth; the teeth and bones are pink, but according to the owner, the urine is not discoloured. The last of these three cases was also seen by Mr. C. T. Nilson, B.V.Sc., Government Veterinary Officer, Bremersdorp, to whom I am indebted for a personal communication concerning it. Unfortunately this bull calf was destroyed by the owner before arrangements were made for the collection of specimens from him. The parents of this calf are blood relations. He is out of a daughter of bull 7015 and sired by the same bull. The mothers of the other 12 cases may be related to the bull, as the bull which is in all probability their sire, is out of the same herd as the sire of bull

7015. This bull, 2 normal cows (mothers of affected animals), 3 normal heifers, 7019, 7021 and 7022 (daughters of the bull) and 4 affected animals (3 steers, 7016, 7017 and 7018 and one heifer, 7023) were purchased by the Division of Veterinary Services and are now at Onderstepoort under observation and being used for breeding purposes (Figs. 1 and 2).*

Fig. 1.—Bull and four affected animals, some daughters of the bull and one cow (extreme right of picture) mother of affected bullock beside her.

Fig. 2.—Bull and four affected animals.

Since this article has gone into press two of the above heifers 7021 and 7022, both daughters of the bull and served by the bull, calved on the 8.3.37. Both calves are heifers. The calf of 7022 is normal but the calf of 7021 has pink teeth. The urine of this animal is not discoloured when it is being voided. No porphyrin bands can be recognised spectroscopically when the urine was examined within 48 hours after the birth of the animal.

Ancestry of bull 7015.—The bull was bred by Mr. B. of S.P.K.P. Eastern Transvaal. He is a pure bred short horn but not registered. The sire of the bull is Br. 2. This bull died in 1929. The owner does not remember seeing anything abnormal in the progeny of this bull, and those of his descendants which were available in 1935, were examined and found to be normal, by Elder, to whom I am indebted for a personal communication concerning this point. The dam, Bilsington Admiral, was never a healthy (?) cow, was in fact a bad doer and even thought to be tuberculous. The owner, however, states that she did not have tuberculosis, but it is not known on what grounds this statement is made. According to the owner animals which did not thrive were met with from time to time in his herd and he particularly remembers a bullock bred from Bilsington Admiral, he was a bad doer and was eventually destroyed. This animal may possibly have had porphyria. Bilsington Admiral died eventually on this owners property. She is supposed to be descended from Texas stock brought into South Africa after the Boer War. It is unfortunate that both the herds from which the dam and the sire of bull 7015 came, are no longer in existence, No case of porphyria could be found in a few herds to which the dispersal of the now non-existent herds could be traced. However, it is hoped that the breeding experiments referred to above, will produce conclusive evidence of the hereditary nature of the condition, already strongly suggested by the foregoing history.

CLINICAL FEATURES.

General.—As already stated the usual history is that affected animals do not thrive. This is particularly noticeable soon after weaning. The animals lose condition and develop rough staring coats. In one animal (7017) there is a lot of coarse, long hair on the head, behind (polar region) between and below (frontal region) the horns. This may not be analogous to hypertrichosis described in some human cases (Günther, Gray) as one sometimes finds normal bovines having this course hair about the head. No definite statement concerning temperament can be made. When the animals arrived here, they were all in rather poor condition and were usually handled with ease. However, one old cow (a normal cow herself), the mother of an affected animal, gave birth to a normal heifer calf, two days after she arrived here. She was so weak that she had to be lifted, but in spite of that she charged anybody who came near her and on account of her weak state she usually went down in the attempt. The animal with the coarse hair (7017) is bled weekly and on one occasion was so nervous and irritable that he charged the assistant and natives in his box. The cow unfortunately died from metritis within a week after arrival here. Although these animals cannot be truthfully described as friendly, these were the only occasions that they showed such a marked degree of irritability and nervousness. In the circumstances one is not justified in definitely attributing to this a pathological significance analogous to nervousness and irritability described in some human cases, especially as occasional normal animals will sometimes also behave like this.

The skin of the first animal examined, was simply one mass of lice, this animal was not regularly dipped, on account of its weak state and this was undoubtedly largely responsible for the gross parasitic infestation. In these circumstances it would not be true to say that such animals are prone to ectoparasitism. If this should be the case, it would not be unexpected on general grounds.

Details of the pulse, respirations and ruminal movements of four affected animals and one normal animal (7022) of the same breeding from the same farm will be found in the accompanying table.

	Pulse.		Respirations.		Ruminal Movements during 5 Minutes.	
	May.	October.	May.	October.	May.	October.
7016.	70	74	36	39	8	10
7017.	66	84	28	23	10	12
7018.	80	72	38	36	11	11
7023.	70	74	36	45	7	9
7022.	72	90	44	36	8	10

No significant functional differences are present. The individual variations are probably due to excitement during handling, etc

These animals were placed on temperatures for long periods. The temperatures recorded vary from 100° F. to 105° F. in affected as well as unaffected animals. These individual variations are probably due to a number of factors, such as handling, antimosan injections and climatic conditions.

Photosensitization.—Lesions are present on the skin which is not protected by hair. Scabs and crusts form around the eyes and nostrils (Fig. 3); there is a nasal discharge and sometimes ulcers

Fig 3.—Animal from which specimens 16786 were collected. Lesions around eyes and nostrils.

on the buccal mucous membrane, particularly of the gums and the lips. In four out of the six animals, which were available for examination, scabs are present on the middle of the back, where the hair parts. In one case the scab measured 12 by 5 cm. (Fig. 4) In at least two other cases the scabs have a similar situation and look almost exactly like the one just described. In one of these cases (7017) scabs are in addition also present behind the horns and around the base of the ears. The hair seems to be efficient protection against the harmful rays of the sun, as lesions are not present even in unpigmented portions of the skin and various parts of the body, which is covered by hair.

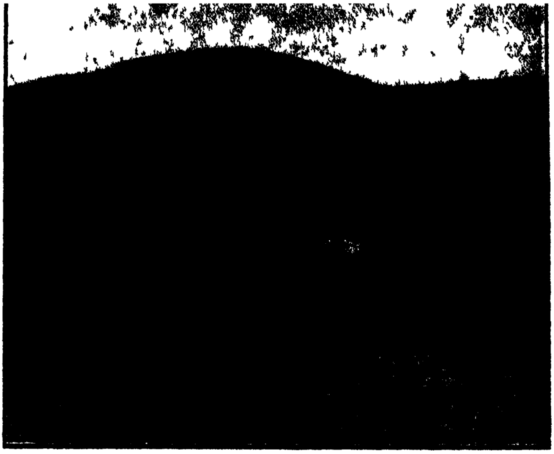


Fig. 4.—Lesions on portions of the skin not protected by the hair. The same animal as in figure 3.

Teeth and bones.—In all cases the temporary and permanent teeth are conspicuously discoloured. Six out of the 13 cases were examined personally. There is no reason why the owner's word should not be accepted for the other cases. The owner's word must also be accepted for the statement that in three out of the 13 cases, pigmentation of the teeth was observed immediately after birth. When the mouth is opened, the general colour impression one gets of the teeth, is that of pink. An attempt was made to reproduce as truly as possible the colour of the incisor teeth in a portion of a formalin preserved mandible (Fig. 5). If the masticatory surface of an incisor tooth is examined with the lingual surface of the tooth towards the observer, the macroscopically clear unpigmented enamel is seen to stand out in sharp contrast to the dentine which is of a brownish pink colour. The labial surface of the tooth on the other hand has a dull brownish pink colour. It is, however, not the enamel which is discoloured, but the dentine which is seen through the somewhat translucent enamel. In the case of the molar teeth, the cement substance on the buccal and lingual surfaces is of a dark red colour. The colour of the table of the tooth varies. The outer enamel is white. The dentine immediately within the outer enamel is brownish pink. Inside this again is the infundibulum, having a white enamel ring, within which the cement substance appears dark red, almost black in colour. Macroscopically therefore, the dentine is pigmented and the cement substance would seen to contain the pigment in even greater concentration, but the enamel is free from pigment. As far as can be determined clinically the pigment distribution in the teeth of the four living animals now at Onderstepoort is similar to that of the case just described. Schmey (1913) also found the enamel of an effected pig to be free from pigment; unfortunately the papers of Colberg and Schenk, who describe pigmentation of the enamel in the teeth of affected bovines, quoted by Schmey, are not available to me. The human cases in whom pigmentation of the enamel is claimed to be present, as described by Garrod and Mackey (1922) and (1926) and by Ashby (1924) have already been referred to.

All the bones are of a deep reddish brown colour (Fig. 6), but cartilages, articular surfaces, ligaments and tendons have a normal colour. The long bones are not uniformly discoloured. This is clearly seen in transverse sections (Fig. 7) where there are alternating darker and more lightly stained rings as described by Teutschlaender (1914) and others previously referred to. Whether this is due to an alternating increased and decreased excretion of porphyrin or to deposition of porphyrin corresponding to alternating periods of growth in summer and absence of this during the winter cannot be stated at the moment. The pathological description of the bones will not be included in this paper, but will be presented at some later date.

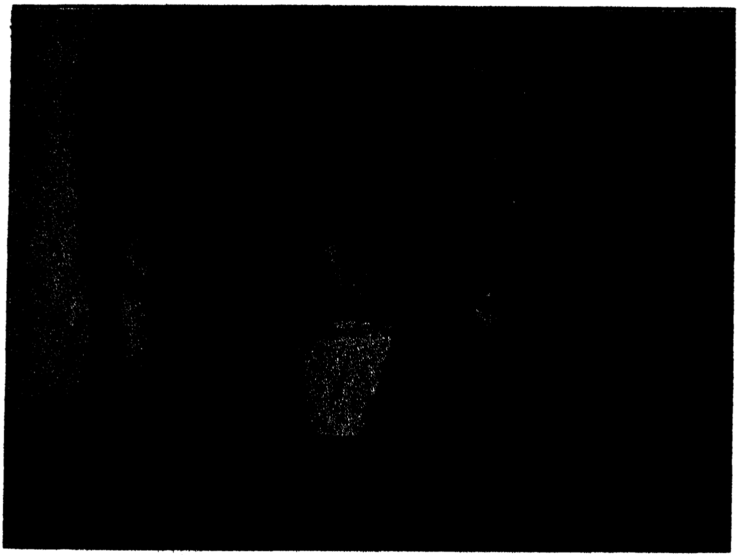


Fig. 8.—Bull 7015. Harness with bucket for the collection of urine.

The Urine.—In no case was a discolouration of the urine observed immediately or soon after birth. A slight discolouration may easily have been missed, when the urine is examined with the naked eye whilst the animal is urinating and yet such a urine may have contained significant amounts of porphyrin. The urine of five cases was available for examination. The urine is not red as occurs

PORTION OF MANDIBLE.

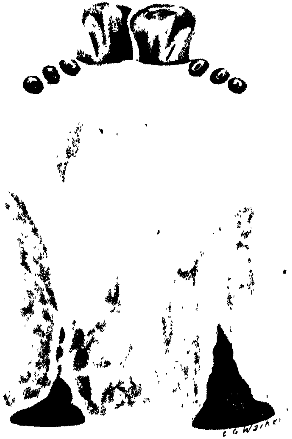


Fig. 5.

SCAPULA.

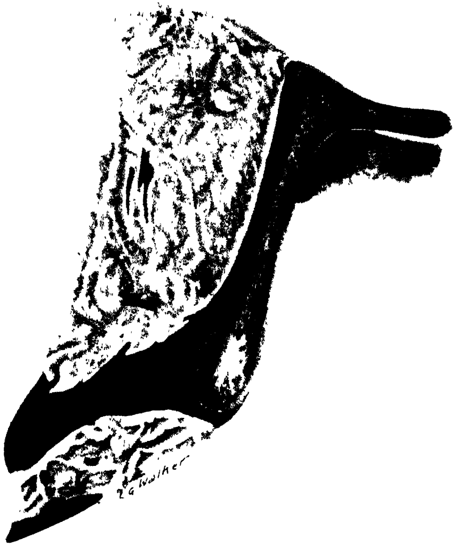


Fig. 6.

METATARSUS.

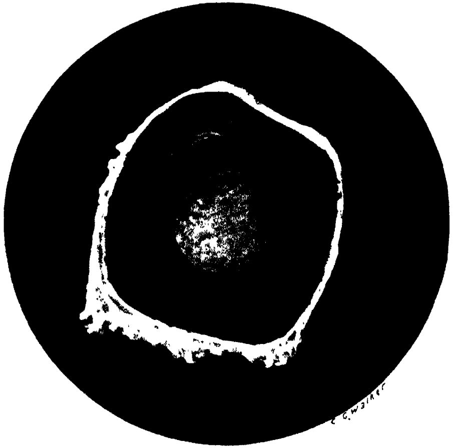


Fig 7.

in cases of haemoglobinuria. The colour of the urine as seen in the urinating animal varies from deep amber to reddish brown. When collected in a flask, even the more lightly coloured urines are seen to have a reddish tinge. For the collection of the urine in males, a special harness was designed, to which a bucket can be fastened and suspended under the abdomen of the animal. (Fig. 8.) The urine should be collected in a glass container as metal is unsatisfactory for porphyrin work. Rimington (1936) found uroporphyrin and coproporphyrin in all five cases. The one case he reports fully in this journal and the urines of the other four cases, together with that of a control unaffected animal, were determined by him as:

	Colour.	*Spectrum.*	*Nature of Pigment.*
7016 Male, 2.3.36	Reddish brown	574·3, 537·8, 500·8	copro- and uro-porphyrin.
7017 Male, 4.3.36	Pinkish brown	574·8, 538·7, 500 2	copro- and uro-porphyrin and some metal complex.
7018 Male, 28.2.36	Deep red	574·4, 538·3, 504·0	copro- and uro-porphyrin and some metal complex.
7023 Female, 4.3.36	Amber-pink tinge	spectrum indistinct	copro- and uro-porphyrin.
7021 Female, 4.3.36	Daughter of bull—normal animal—urine normal appearance—no porphyrin present.		
7015 Male	Bull—yellow urine—turbid, undetermined protein present—but no porphyrins present.		

At this stage it is necessary to mention that these animals were unfortunately also infected with Schistosomes. Le Roux (1929) quotes a number of authors (Stiles, 1898), (Piller 1915), (Kaup, 1918), who describe haematuria in cattle infected with schistosomiasis. Haematuria is not present in any of these cases, neither is there any discolouration of the urine in other animals infected with bilharzia, but not affected with porphyrinuria, from the same farm. Some of the bilharzia infected animals were treated with tartar emetic, but as the use of this drug did not completely succeed in curing the animals, antimosan was used, with apparently greater success. (For details of treatment see tables 1 to 5). The excretion of porphyrins continued for some months after completion of the treatment. In the circumstances there would seem to be very little doubt that the excretion of porphyrins in the urine of these animals cannot in any way be associated with the bilharzia infection which was present. To make absolutely certain of this point, it would be necessary to examine the urine of bilharzia cases in which haematuria is present, for porphyrins. Such cases have unfortunately up to the present not been available for examination. At the moment no opinion can be given as to whether there may be a variation in the daily amount of porphyrin excreted by these affected animals.

HAEMATOLOGY.

Of the four clinically affected animals, two young bullocks (7017 and 7018) seem to have the condition in a more severe form. A haematological examination of these two cases was made weekly and the other two (7016, young bullock, and 7023, heifer) were examined bi-weekly. In addition a bi-weekly examination was made of another normal animal (7022, heifer) of the same breeding, of more or less the same age and from the same farm. This animal is being regarded as a control. Details concerning these haematological observations will be found in the accompanying graph and tables (7018—graph 1 and table 1; 7017—table 2; 7016—table 3; 7023—table 4; 7022—table 5).

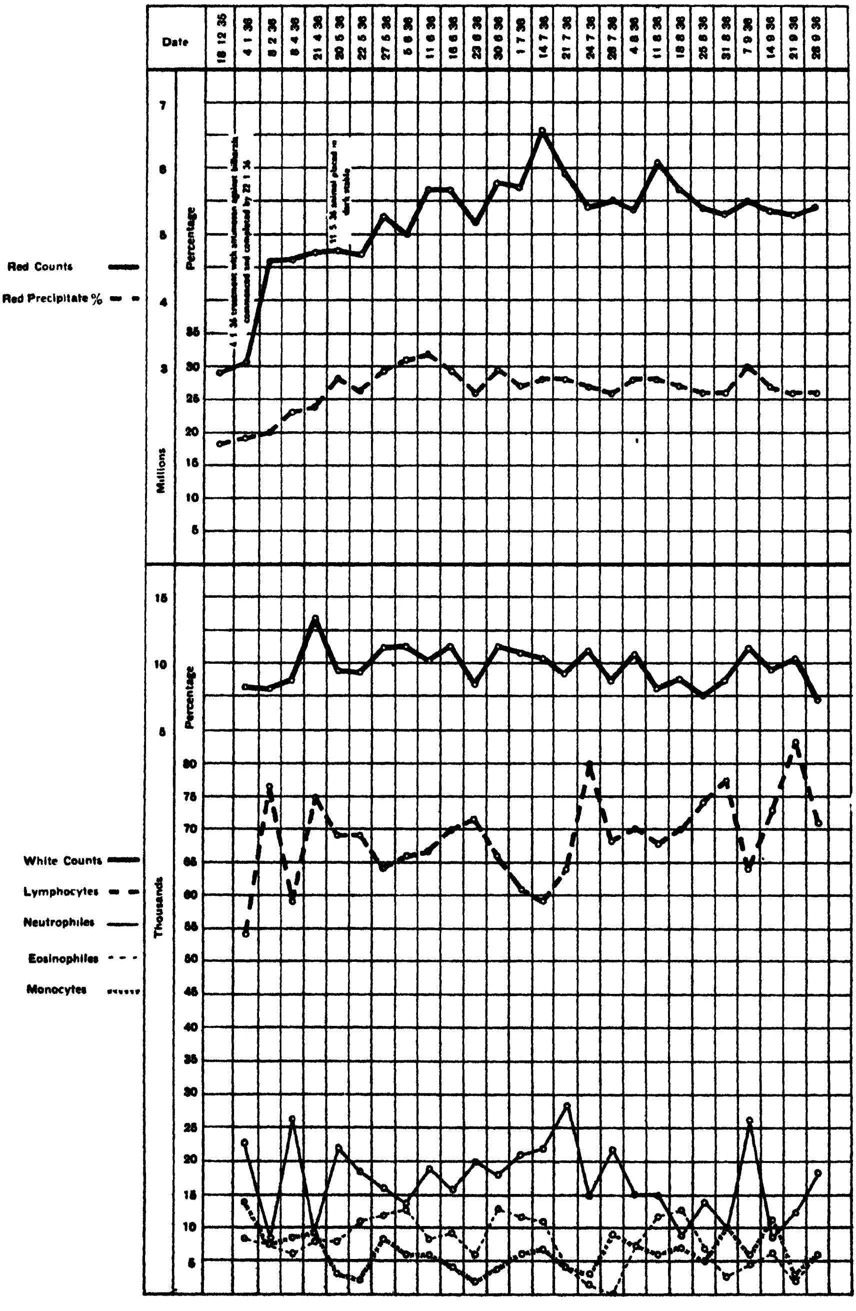
These observations lose a great deal in value on account of the fact that even as late as the 20th October, 1936, bilharzia eggs were found to be present in the faeces of animal No. 7017, indicating that the antimosan treatment was not successful in completely destroying the bilharzia infection in all the animals. However, in the case of 7018 bilharzia eggs were never found in its faeces on the two or three occasions that an examination was made subsequent to treatment, the last examination being made on the 20th October, 1936. Even though this is the case, the disturbing possibility that bilharzia infection may be a contributary factor in the production of any haematological changes which may be present, cannot be ignored. In any case no marked morphological changes are present in the red cells of any of these animals, with the exception of No. 7017, in which case the anaemia is largely if not entirely due to an acute attack of anaplasmosis.

7018.—*Young ox, 2-3 years old.*—When the animal arrived here in December, 1935, this animal was undoubtedly the poorest of the lot. His red counts were 2·9 million per c.c. The red counts increased to 4·6 million per c.c. a month after the animal was treated for bilharzia and then remained at this level for a period of 3 months. (See graph 1). The animal was then placed in a dark stable (11.8.36) in order to see to what extent further improvement in its blood picture and general condition will take place if it is protected against the harmful rays of the sun. The red counts did actually increase, until they finally fluctuated around about the 5·5 million level. Whether this is due to the absence of haemolysis as a result of the protection afforded by the dark stable cannot be stated at the moment, but it is hoped to clear up this point at a later date.

The blood relations of this animal, more or less of the same age and running in a small paddock in which they can freely move about, have counts which vary from 6-8 million per c.c. of blood. Canham (1930) has shown that animals confined to pens will have red counts which are 1 to 3 million per c.c. of blood less than those of similar animals running in the veld. In an ordinary loose box, in which animal 7018 is always being kept, he cannot get any exercise at all and it is believed that his counts of 5·5 million per c.c. of blood can in the circumstances be regarded as normal for him.

No well marked morphological changes are present in the red cells of this animal. Occasional normoblasts and occasional punctuate basophiles were recognised, but not in sufficient numbers to regard them as a significant disturbance of the blood picture. It is conceivable that if the exposure of the animal to the sun had been continued, a more severe degree of anaemia may have developed, when more pronounced morphological changes in the red cells may have taken place.

GRAPH I.


Date
18 12 35
4 1 36
8 2 36
8 4 36
21 4 36
20 5 36
22 5 36
27 5 36
5 6 36
11 6 36
16 6 36
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30 6 36
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21 7 36
24 7 36
28 7 36
4 8 36
11 8 36
18 8 36
25 8 36
31 8 36
7 9 36
14 9 36
21 9 36
28 9 36
4 1 36 treatment with antimosan against bilharzia commenced and completed by 22 1 36
11 5 36 animal placed in dark stable
7
6
5
4
3
Millions
Percentage
35
30
25
20
15
10
5
Red Counts
Red Precipitate %
15
10
5
Thousands
Percentage
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
White Counts
Lymphocytes
Neutrophiles
Eosinophiles
Monocytes

TABLE I.

Affected Bovine 7018—Young Ox—2 Permanent Incisors December 1935.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
18/12/35.......	2·9	—	5 2	—	—	—	—	—	—
4/1/36.........	3·0	19	7·7	54	14	23	8	1	Anisocytosis, jolly bodies and punctate basophilia rare.
18/2/36........	4·6	20	7·7	77	7	8	7	1	Anisocytosis, jolly bodies and punctate basophilia rare.
8/4/36.........	4·6	23	8·5	59	28	6	6	1	Anisocytosis, jolly bodies and punctate basophilia rare.
21/4/36........	4·7	24	13·3	75	9	9	7	0	Cells normal.
20/5/36........	4·6	28	9·4	68	3	22	7	0	Cells normal.
22/5/36........	4·7	26·5	9·3	69	2	18	11	0	Cells normal.
27/5/36........	5·3	29	11·2	64	8	16	12	0	Cells normal.
5/6/36.........	5·0	31	11·3	65	6	14	13	2	Punctate basophilia very rare, Theileria mutans rare.
11/6/36........	5·7	32	10·1	67	6	19	8	0	Cells normal.
16/6/36........	5·7	29	11·4	70	4	16	9	1	Occasional punctate basophilia.
23/6/36........	5·2	26	8·1	72	2	20	6	0	Cells normal.
30/6/36........	5·8	29	11·5	66	4	17	13	0	Occasional punctate basophilia.
1/7/36.........	5·7	27	10·8	61	6	21	12	0	Occasional punctate basophilia.
14/7/36........	6·5	28	10·2	59	7	22	11	1	Occasional punctate basophilia.
21/7/36........	5·9	28	9·5	64	4	28	4	0	Occasional punctate basophilia.
24/7/36........	5·4	27	11·1	80	3	15	2	0	Occasional punctate basophilia.
28/7/36........	5·5	26	8·7	68	9	22	0	1	Occasional punctate basophilia.
4/8/36.........	5·4	28	10·4	70	7	15	7	1	Occasional punctate basophilia.
11/8/36........	6·1	28	7 9	67	6	15	12	0	Cells normal.
18/8/36........	5·7	27	8·7	70	7	9	13	1	Young forms of eosinophiles and one or two eosinophile myelocytes, otherwise normal.
25/8/36........	5·4	26	7·5	74	5	14	7	0	Cells normal.
31/8/36........	5·3	26	8·8	77	10	10	3	0	Occasional Theileria mutans otherwise normal.
7/9/36.........	5·5	30	11 4	64	6	25	5	0	Occasional punctate basophilia.
14/9/36........	5·4	27	9·5	73	11	8	6	2	Occasional punctate basophilia.
21/9/36........	5·3	26	10·2	83	3	12	2	0	Cells normal.
28/9/36........	5·4	26	7·2	70	6	18	6	0	Occasional normoblasts, one eosinophile myelocyte—cells normal.

Animal was found to be infected with bilharzia—was given 25–30 cs. of 6·3 per cent. antimosan intravenously until 10 injections were given. Treatment commenced 4 1/36 and completed 22/1/36. Animal was placed in dark stable as from the 11/5/36—no parasites seen in faeces on 20/10/36.

The improvement in the blood picture was not nearly as striking as the improvement in the general appearance and condition of the animal. (See figs. 9 and 10). His weight increased from 630 lb. at the time he was placel in a dark stable (11.5.36) to 970 lb. on the 26.10.36 (5½ months in dark stable) at the rate of more or less 2 lb. per day. There are possibly a number of factors which are responsible for this remarkable general improvement in condition, etc. By eliminating the photosensitizing factor, the animal is now in a state of greater complacency and is in a position not only to relish its food, but will probably also eat more of it. Being alone in the stable, it is no longer necessary for him to compete with its fellows, in collecting his share of the food and may possibly on this account eat more and in greater comfort. The food he is getting is otherwise practically the same as he was getting before, except that a small daily ration of green food was prescribed. in order to avoid the possibility of introducing a vitamin deficiency factor in the absence of sunlight. The improvement in his blood picture is probably also a factor, but here again, it cannot at present be stated whether the elimination of the harmful rays is directly (absence of haemolysis) or indirectly responsible for this.

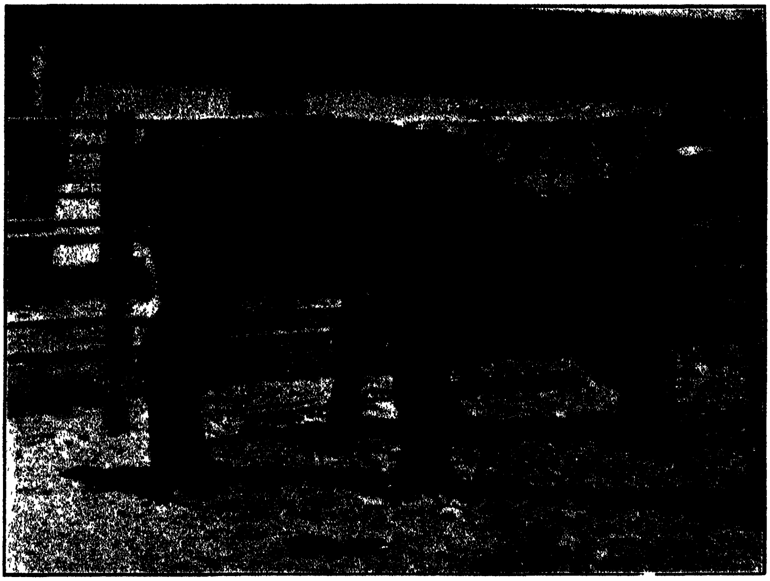

Fig. 9.—Animal 7017; 11.5.36: weight, 630 lb.

The scabs around nose and eyes have disappeared and the scab on the back is completely healed. At the moment the general state of health of the animal is excellent, but the porphyrin excretion continues, and the urine remains discoloured. It is true that the urine is no longer as darkly coloured as it was some 5 month ago, before the animal was placed in the dark stable. At that time the colour of the urine was described as deep red; to-day the colour is deep amber with a brownish tinge, but still very much darker in colour than the urine of the normal animal. It is difficult to avoid the conclusion that the changed enviroment, viz. the dark stable is responsible for this and the brown pigment in the urine is mainly due to the effects of the rays of the sun on the photosensitive porphyrin animal.

On examining the plasma of this animal it was found to be a deep yellow colour. It gave a negative direct and indirect van den Berg's reaction but on further examination the yellow colour was found to be due to the presence of lipochromes.

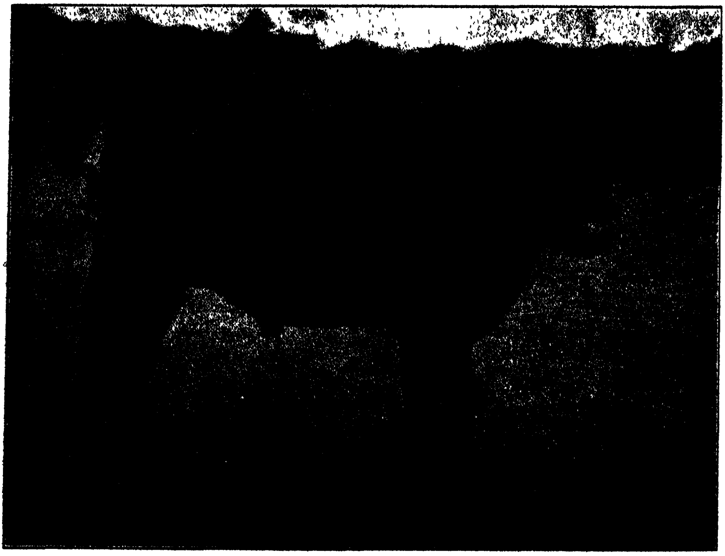

Fig. 10.—Same as figure 1; 26.10.36; weight, 970 lb.

The total number of leucocytes fall within normal limits, but the neutrophiles would seem to be decreased in number. At times this decrease is due to a relative increase in the lymphocytes, but on some days there would seem to be a relative increase in the monocytes and the eosinophiles. In several instances eosinophile myelocytes were recognised. The nuclei of quite a number of other eosinophiles have the appearance of young forms. The presence of these cells suggest a certain amount of immaturity. The evidence (negative faeces examination) suggests that the animal is now free form bilharzia, but in view of the fact that some of the other animals similarly treated are still definitely infected and further that even higher eosinophile counts are recorded for the control animal 7022 (table 5) which is free from porphyrinuria, but was also infected with bilharzia, one cannot at the moment attribute the presence of the eosinophiles to either a possible bilharzia infection or to the porphyrinuria, with any degree of certainty.

TABLE 11.

Affected Bovine 7017—4 *Permanent Incisors December* 1935.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
18/2/36........	4·0	26	14·1	52	1	39	4	4	—
23/4/36........	4·0	26	12·2	63	6	23	7	1	Anisocytosis, jolly bodies, slight polychromasia, occasional normoblasts.
20/5/36........	3·8	29	6·8	67	6	21	5	1	Anisocytosis, punctate basophilia rare.
27/5/36........	2·4	19	15·2	56	10	24	0	0	Anaplasma marginale numerous, marked polychromasia and punctate basophilia.
5/6/36.........	2·6	25	24·1	51	2	45	1	1	Anaplasma, punctate basophilia and polychromasia, occasional normoblasts showing basophile punctation.
11/6/36........	3·7	28	10·1	64	5	30	0	1	Anaplasma infrequent, punctate basophilia slight.
16/6/36........	3·5	27	10·5	48	17	33	1	1	Punctate basophilia rare—monocytosis.
23/6/36........	3·4	27	13·0	45	15	31	9	0	Anaplasma infrequent, Theileria mutans frequent, polychromasia and punctate basophilia monocytosis.
30/6/36........	3·2	22	14·3	49	7	40	4	0	Theileria mutans frequent, punctate basophilia slight.
1/7/36.........	3·1	21	11·2	37	12	44	7	0	Theileria mutans and anaplasma—polychromasia.
14/7/36........	3·5	24	11·9	48	20	31	2	0	Theileria mutans numerous—punctate basophilia frequent.
21/7/36........	3·3	23	14·3	41	10	48	2	1	Theileria mutans numerous—punctate basophilia normoblasts frequent.
24/7/36........	3·4	22	12·8	55	13	30	2	0	Theileria mutans numerous—punctate basophilia normoblasts frequent.
28/7/36........	2·9	22	9·3	67	9	15	10	0	Theileria mutans numerous—punctate basophilia normoblasts frequent.
4/8/36.........	3·5	24	17·8	44	10	41	5	1	Jolly bodies—punctate basophilia normoblasts. showing punctate basophilia.
11/8/36........	3·7	22	10·9	51	13	18	18	0	Punctate basophilia—normoblasts Theileria mutans.
18/8/36........	3·4	22	14·0	45	12	31	12	0	Theileria mutans rare, no punctate basophilia seen.
25/8/36........	3·3	23	11·0	66	9	18	7	0	Normoblasts Theileria mutans punctate basophilia rare.
31/8/36........	3·3	24	11·9	59	11	25	5	0	Occasional normoblasts punctuate basophilia rare.
7/9/36.........	3·3	24	11·0	69	7	12	12	0	Occasional anaplasma punctate basophilia absent Theileria mutans.
14/9/36........	3·2	23	9·9	—	—	—	—	—	—
20/9/36........	3·2	—	12·4	50	7	41	2	0	Punctate basophilia rare normoblasts present.
28/9/36........	3·3	24	9·4	65	6	28	2	0	Normoblasts present.

Animal was treated for bilharzia by giving five intravenous injections of antimosan commencing 18/2/36 with 20 ccs. and thereafter 30 ccs. every second day until the course of treatment completed on the 26/2/36.

On 26/5/36 animal very sick. Anaplasmosis diagnosed and injected intravenously ·75 gms. Mercurochrome and 40 gms. glucose in 200 ccs. saline. As bilharzia treatment was not successful the animal was given 10 injections of antimosan every second day commencing 23/4/36 with 20 ccs. then 25 cc. and thereafter 30 cc. until the course of treatment was completed. Even this did not completely cure the animal as bilharzia eggs were found to be present in the faeces on 20/10/36.

TABLE III.

Affected Bovine 7016—*Young Ox—No Permanent Incisors December* 1935.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
18/12/35.......	8·7	28	17·5	—	—	—	—	—	—
18/2/36........	6·7	32	11·8	69	8	17	6	0	Several eosinophile myelocytes present otherwise cells normal.
22/4/36........	6·0	32	13·9	62	4	28	5	1	Cells are normal.
3/7/36.........	6·1	31	14·3	70	7	19	3	1	Occasional Theileria mutans—cells normal.
27/7/36........	6·9	35	15·1	78	6	11	5	0	One eosinophile myelocyte counted otherwise cells normal.
11/8/36........	7·0	38	14·2	79	8	9	4	0	Cells normal.
25/8/36........	8·0	39	13·7	80	1	16	4	0	Nuclei of cells contracted, darkly staining and monocytes may have been counted as lymphocytes.
7/9/36.........	6·8	38	12·8	77	5	15	3	0	Occasional Theileria mutans otherwise normal.
22/9/36........	6 8	38	13·5	77	3	14	6	0	Anisocytosis marked, some very large red cells present.

Owing to bilharzia infection, the animal was given 5 intravenous injections of 6·3 per cent. solution of antimosan every alternate day, commencing with 20 c.s. on the 18/2/36 thereafter 25 c.s. until the course of treatment was completed on the 26/2/36. Six weeks after completion of the treatment, a badly infected animal which was similarly treated, was still found to harbour parasites and it was decided to give 10 intravenous injections of antimosan every alternate day commencing on the 23/4/36 with 25 c.s. and using thereafter 30 c.s., until the course of treatment was completed on the 13/5/36.

TABLE IV.

Affected Bovine 7023—Heifer—No Permanent Incisors December, 1935.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
18/12/35.......	4·6	25	8·1	—	—	—	—	—	—
18/2/36........	6·5	30	9·3	61	8	16	15	0	Occasional Theileria mutans. Cells normal.
3/7/36.........	6·7	37	13·0	81	3	8	7	1	Occasional normoblasts nuclei pycnotic and monocytes may have been counted as lymphocytes.
27/7/36........	7·1	40	8·6	62	13	17	7	1	Cells normal.
18/8/36........	6·8	37	7·7	75	7	13	5	0	Occasional Theileria mutans. Cells normal.
25/8/36........	7·5	36	12·3	72	6	19	3	0	Cells normal. One cell resembling neutrophile myelocyte seen.
7/9/36.........	6·9	37	8·2	72	3	18	7	0	Cells normal. One cell resembling neutrophile myelocyte seen.
22/9/36........	8·5	41	8·1	68	5	23	4	0	—

Treated for bilharzia by giving 5 intravenous injections of antimosan (6·3%) every second day commencing on the 18/2/36 with 20 ccs. and thereafter 25 ccs. until course of treatment completed. As treatment was not successful 10 injections were given every second day commencing on the 23/4/36 with 20 ccs. and thereafter 25 ccs. until course of treatment completed on the 13/5/36.

TABLE V.

Bovine 7022—Heifer four Permanent Incisors December, 1935.

Normal animal but half sister to porphyrin animals.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
4/1/36.........	6·3	33	7·3	56	4	21	19	0	Occasional Theileria mutans.
18/2/36........	6·4	34	8·7	57	6	19	16	2	Occasional Theileria mutans present.
22/4/36........	7·5	38	6·1	60	8	25	5	2	Cells normal.
7/5/36.........	—	—	—	71	1	23	5	0	Numerous pirop bigem and injected 4 ccs. acaprin intravenously—no signs of anaemia.
18/5/36........	—	—	—	37	4	58	1	0	Few pirop. bigem. present.
27/7/36........	7·7	39	12·5	66	7	17	10	0	Cells normal.
11/8/36........	6·8	37	9·8	77	2	11	10	0	Occasional Theileria mutans. Cells normal.
25/8/36........	7·7	41	10·2	75	5	9	11	0	Cells normal.
7/9/36.........	7·0	40	11·9	72	3	15	10	0	Cells normal.
22/9/36........	7·9	44	5·6	74	0	17	9	0	Cells normal.

Animal was treated against bilharzia with Tartar Emetic (4% solution) every second day intravenously into the ear vein. The first day 7 ccs. were given then 10 ccs. and thereafter 15 ccs. until a course of 10 injections was completed on the 8/1/36. This was not successful and a course of 10 injections of 30 ccs. of Antimosan (8% intravenously) was commenced on the 23/4/36 and completed on the 13/5/36. Animal developed acute natural Redwater on the 17/5/36 and was successfully treated with 4 ccs. of Acaprin.

On some days the monocytes are decidedly increased in number and on the whole the monocytic counts in all four porphyrinuria animals run on a rather higher level than those of the control animal 7022 (table 5). Even though this is the case, Wirth (1931) records the variations in the number of monocytes as being from 3 to 10 per cent. and although counts beyond the 10 per cent. limit are repeatedly recorded, one cannot at this stage, remembering at the same time also, the bilharzia factor, regard a monocytosis as a significant reaction in cases of porphyria. However, the possibility of this should not be lost sight of, particularly in view of the statement by Mackey and Garrod already quoted, that there would seem to be an increase of cells described by them as endothelials in the case of a boy examined by them.

7017. *Young ox. 2-3 years old.*—The effects of porphyria are well marked in this animal. It was intended to keep him exposed to the sun, as a control to 7018, when this animal was placed in a dark stable. Unfortunately animal 7017 developed an acute attack of anaplasmosis (Gallsickness). He was treated with mercurochrome on the 26.5.36 and in order to save the life of the animal the original idea, to keep him exposed to the sun, had to be abandoned and he was also continuously kept in a dark stable. In spite of getting a course of ten injections of antimosan, during April and May, 1936, bilharzia eggs were found to be present in his faeces on the 20.10.36. When this animal arrived here his red cells were also found to be decreased in number. (4 million per cm.). He then had porphyrinuria, as well as being infected with bilharzia. The five injections of antimosan given to the animal during the period 18.2.36 to the 26.2.36, made no appreciable difference to the excretion of bilharzia eggs in its faeces, and this may have been responsible for the continued low counts. Neither did the further course of ten injections of antimosan (see table 2) during the period 23.4.36 to the 13.5.36 succeed in improving the red counts, which remained more or less stationary until the 3.5.36, and were further decreased on the 27.5.36. However, this further decrease is undoubtedly due to the anaplasmosis infection, which almost certainly took place from infected ticks, which the animal must have picked up whilst he was being moved forwards and backwards to receive the antimosan injections in a crush some distance away from its quarters during the period 23.4.36 to the 13.5.36. In view of the anaplasmosis and bilharzia complications, a further discussion of the blood picture of this animal in relation to porphyrinuria will be of no value at the present time.

Animals 7016, 7023 *and* 7022 (*control*).—The neutrophiles seem to be on the low side in all three animals. In animal 7022 (free from porphyrinuria, but also infected with bilharzia) the eosinophiles run on a higher level, than in the case of the other two animals. In none of these animals do the monocytes call for special comment. No morphological changes are present in the red cells. Morphological changes in the red cells of the control animal 7022, may have been present as a result of an acute attack of redwater (Piroplasma bigem.) during the period 18.5.36 to 27.7.36 when an examination of its blood was not made. Theileria mutans is a parasite which is present in nearly every normal bovine in certain areas in South Africa and is not known to produce any disturbances.

PATHOLOGY.

Specimens from two animals were available for examination. These animals were both killed in Swaziland on the owner's farm. In view of the presence of bilharzia infection in the animals which came from that farm and that bilharzia eggs were definitely found to be present in sections cut from the liver of one of these animals, it is exceedingly likely that both these animals were infected and one would consequently not be justified in regarding the various pigments present in the organs as being due to the porphyrinuria alone. It is nevertheless thought desirable to give a short description of the appearance of the pigment stained with various stains, with the hope that this may serve as a comparison for the histological description to be presented at some later date, of an animal which is free from bilharzia and which has been kept in the dark stable, to eliminate any secondary factors such as a possible haemolysis, which may take place when the porphyria animal is exposed to the sun. In view of the fact that the sections from these animals were not examined by the fluorescence microscope, any analogous reference to the pigments present, in terms of Borst and Köningsdroffer's description as P_1, P_2, P_3, P_4, P_5, must be regarded as speculative.

Specimen 16786, bovine, 2 years and 4 months old, killed on the farm. The biochemical details of the pigments in bone, urine, blood and other organs are described by Rimington elsewhere in this journal.

Kidney.—This organ is darker in colour than normal; its surface has a mottled appearance; on section marked striation is seen to be present in the cortex; dark lines alternating with greyish ones. The medulla is of a pinkish colour. The bladder contains a small amount (50 c.c.) of clear urine, having a port wine colour with a slight brownish tinge.

Microscopic examination.—Haem. Eosin. most of the pigment is in the form of yellowish brown granules (brown with v. Giesen) in the cells of the renal tubules of the cortex and perhaps also of the boundary zone, but very little, if any, is seen in the straight and collecting tubules of the medulla. Bigger conglomerate masses of brownish yellow (brown with v. Giesen) pigment are also present, mainly in the cortex and boundary zone but to a less extent in the medulla. Neither of these pigments are present in the structures of the glomerulus. The finely granular yellowish brown pigment described by Borst and Köningsdroffer in the endothelial cells of blood vessels, is probably also present, but was recognised with difficulty and then only with the high magnifications under oil. A structureless pink (brown with von Giesen; greenish blue, but sometimes the colour is very faint with Berliner Blue) staining substance, is present in the lumen of the renal tubules. This substance is sometimes also present in Bowman's capsule.

With Berliner Blue, there is a considerable variation in the staining reactions of the pigment, which appears as: (1) small granules of varying size in the cells of the tubules. Most of them are brown, but some are of a greenish blue colour; (2) very small granules, which can only be seen with a 2 m.m. oil immersion lens. They are mostly of a yellowish brown colour, but occasionally they are of a greenish colour. They occur in cells, in the walls of the tubules. Practically all the cells of some tubules may contain this pigment, but the collecting tubules of the medulla contain much less of this pigment than do tubules in other parts of the kidney; (3), (*a*) big conglomorate brown pigment masses in tubular cells, (*b*) pigment masses of similar size, etc., but staining of a greenish blue colour, (*c*) what appears to be an intermediate stage between (*a*) and (*b*), where the pigment has only a slight greenish tinge.

Sudan III.—The pigment granules are brown and the conglomerate pigment masses are darker in colour. The granules and bigger pigment masses may occur in the same tubule and even in the same cell. The structureless material in the tubules does not stain differentially with the fat stain. It has a faded bluish colour. In Bowman's capsule a brownish staining pigment is sometimes seen to be present. In some glomeruli, pigment granules are only seen in the neck, but all other structures of the glomerulus are free from pigment. With the lower magnifiication no pigment granules can be seen in the tubules of the medulla, but with a 4 m.m. dry lens, a number of tubules in the medulla are seen to contain very fine granules. In addition to the pigmentation of the kidney, there is fairly well marked fibrosis of the organ. From the other animal kidney specimens were not available for examination.

Diagnosis: marked pigmentation-fibrosis.

Liver: 2 animals, specimens 16786 *and* 16719. The stern cells are prominent and are loaded with pigment. The pigment is mostly in the form of granules, some of which stain of an almost lemon yellow colour, whilst others stain of a yellowish brown colour, with haemalum-eosin and with Sudan III. With von Giesen the granules stain brown, less frequently similarly staining conglomerate pigment masses are seen to be present, probably also in stern cells. With Berliner Blue very little iron staining pigment can be indentified under the lower magnifications; but with a 2 m.m. oil immersion lens one can easily recognise pigment which is in the form of fine blue lines or rings, especially in the sinusoids. With the same magnification one can also recognise bluish staining pigment in the liver cells themselves.

In some of the bigger vessels of the liver (specimen 16786) ovoid structures are present. They have a definite outer wall, within which cellular elements can be recognised. These structures are regarded as eggs of bilharzia parasites and in the circumstances one cannot be sure to what extent some of the pigment present may not be due to these parasites. In Giemsa stained sections, numerous eosinophiles can readily be recognised along the interstitial tissues, but some are also present in the sinusoids. Remembering the bilharzia infection one is not justified in regarding the eosinophiles as being significantly associated with the presence of porphyrins.

Diagnosis: pigmentation and bilharzia infection.

Lymphatic glands 16786 *and* 16719.—In freezing sections the the distribution of the pigment, mainly in the reticulum of the gland is well shewn. In embedded sections, the dominating pigment is seen to be present in the reticulum cells as fine granules, having a yellowish brown colour (P_2). The lymphoid follicles seem to be free of pigment, except that in the germ centres of some of them one may find structureless masses 5 to 10 times the size of the red cell, staining of a lemon-yellow colour with van Giesen. Occasionally masses of yellowish brown pigment (P_1) are seen in the lymphoid cords. In addition to the pigments just described one sometimes finds cells which are loaded with fine granules staining of a purplish colour with Giemsa, in the lymphoid cords.

Berliner Blue.—Most of the granular pigment in the reticulum cells stains of a greenish yellow colour, but a well defined iron reacting pigment as described by Borst and Köningsdroffer for Petry, could not be recognised. With a 2 m.m. oil immersion lens one does sometimes see blue rings or blue lines in or about the reticulum cells, but except for the occurrence of very occasional blue granules, this is the only evidence of stainable iron in the lymphatic glands. In places an increased number of eosinophiles would seem to be present.

Spleen—specimens 16786 *and* 16719. *Haemalum eosin.*—Large amounts of pigment are present. The granular (P_2) type of pigment is present in relatively small amounts. Most of the pigment is in the form of big masses (P_4) but in some cases it is also coarsely granular. Close to the pigment masses one nearly always sees a nucleus but the type of cell in which the pigment occurs, cannot be so clearly recognised as in the case of the liver. The pigment varies in colour from a light yellowish brown to a dark brown or almost black colour. In some of these pigment masses, one sometimes sees with a 2 m.m. lens small darkly staining pigment granules.

Berliner Blue.—In the pulp one recognises: (1) cells containing big blobs of pigment staining yellowish brown, with small darkly staining granules within the bigger pigment mass; (2) cells containing granules staining yellowish brown; (3) cells containing pigment blobs staining greenish blue; (4) cells containing granules staining greenish blue. In some cases the pigment stains between green and yellow and one has the impression that the yellowish staining pigment is being changed or converted into the stainable haemosiderin. The distribution of the pigment is well shewn with this stain. The malpighian bodies stand out prominently and do not contain any iron-staining pigment, but they do sometimes show the presence of fairly big, round, lemon yellow, pigment masses.

In the pulp one sees what can be most suitably described as *pigment rings.* On careful examination under high magnification the space enclosed by the rings which consist of bluish pigment is seen to be occupied by a red cell. It almost seems as if the blue ring represents pigment which is lying free between the red cells and which has in some way become absorbed to the periphery of the cells. In order to find out if the red cells of porphyrin animals contain any stainable iron, smears were made from animal 7018 but no iron could be recognised after staining with Berliner Blue. In some cases it is not possible to recognise with certainty what is present in the space enclosed by the rings. The pigment is not only present in the form of rings, but occurs sometimes in the form of irregular lines, also in small amounts in the trabeculae. Although no undoubted phagocytosed red cells were encountered in the spleen, one cannot entirely ignore the possibility that some of the large pigment masses may be the remains of phagocytosed red cells. In the pulp eosinophiles are numerous.

Lung. Only one specimen, 16786.—There is only slight pigmentation of the lung. Pigment, in the form of fine granules (P_3) is present in the interalveolar walls. The granules stain yellowish brown with Haem. eosin and brown with Berliner Blue. Occasionally pigment masses are seen in cells as well as in the interalveolar walls. These stain blue with Berliner Blue, but dark brown granules can be recognised under high magnification in the substance of this bluish structureless mass.

Myocardium.—A small amount of non iron staining coarsely granular pigment is present in the interstitial tissues. It stains of a lemon yellow colour with Berliner Blne and of a dark yellowish brown colour with Haem. eosin.

Muscle.—No pigment was recognised.

A notable ommission in the pathological description is that of the bones. During 1935 Sir Arnold Theiler signified his willingness to collaborate in the further work which was contemplated and he would have been resposible for the pathological study of the bones. His unexpected death unfortunately broke this collaboration. It is a tragedy that an authority of his standing, on osteopathology, could not complete the work, for the successful execution of which he was brilliantly equipped.

PATHOGENESIS.

Nothing fundamentally new and original can be contributed at this stage. Animals 7016 and 7023 seem to be affected to a mild degree only and consequently do not afford a clue as to the possible sequence of events. Animal 7017, in spite of treatment, still harbours bilharzia parasites and has in addition only recently recovered from an acute attack of anaplasmosis (Gallsickness). On account of these complications this animal is not at the moment a suitable subject for the discussion of porphyrin pathogenesis. None of these objections can be raised against the use of animal 7018 for this purpose. One should, however, remember the possibility that a negative faeces examination for bilharzia eggs, even though it is repeated 2 or 3 times, as was actually done in this case, does not necessarily mean that the animal is free from infection.

The catabolism (Abbau) theory and the anabolism (Aufbau) theory of porphyrin genesis are exhaustively discussed by Günther, Borst and Königsdörfer and others. It is consequently unnecessary to present here details of all the claims for and against these theories, but briefly stated the theories are:—

(1) *Catabolism (Abbau) theory.*—During the catabolism of the haemoglobin molecule porphyrins are formed before the final degradation to bile pigments. Chemically this is possible but it has not yet been established that this occurs in vivo, neither has it up to the present been possible to split the haemoglobin molecule to bile pigments, through a porphyrin stage in vitro.

(2) *Anabolism (Aufbau) theory.*—Here it is postulated that the synthesis of the haemoglobin molecule from pyrrol bodies passes through a porphyrin stage. Chemically this is not only possible but Fischer (1931) was actually successful in synthesising the haemoglobin molecule through a porphyrin stage, by coupling the resulting haematin with native globin. Furthermore, Borst and Königsdörffer find that porphyrins are present under normal physiological conditions in serum, liver, kidney and bones of young embryos. In the six months old human embryo, porphyrin containing cells are only present in the liver, but not in the blood and bone marrow. A proportion of the erythroblasts in the blood islands occurring in the liver of the 4-6 months old human foetus show a weak porphyrin fluorescence, and at the same time faint haemoglobin staining, with recognisable haemoglobin absorption bands. This is interpreted to mean that porphyrins form a link in the normal haemoglobin synthesis.

In their pathological investigations, they find erythro- and megalo-blasts containing porphyrin in the bone marrow, spleen and blood of humans and equines suffering from pernicious anaemia. In the bone marrow of Petry they describe erythrophagoblastosis (if one may be permitted to use this term where phagocytosis of erythroblasts occurs as against erythrophagocytosis, where the erythrocytes are phagocytosed). Porphyrins are present in phagocytosed as well as in free erythroblasts, although they also find some erythroblasts, which do not show any porphyrin fluorescence. This they regard as evidence that at least a portion of the haemoglobin synthesis occurs normally. The other portion stops short at the porphyrin stage indicating the persistence of a foetal process, in post foetal life.

The question now arises as to which of these two theories can satisfactorily explain the recorded observations on these animal cases. For reasons already stated, animal 7018 is the only one worth discussing at this stage. When this animal arrived here, its red counts were 2·9 milloin per c.c. Being infected with bilharzia, antimosan treatment was applied and within a month after treatment the red counts increased to 4·6 million per c.c. and remained at this level for some months. He was then placed in a dark stable and the red counts further increased to the 5·5 million level. This is being regarded as a normal count for this animal under conditions of inactivity in a loose box. No well marked morphological changes are present in the red cells or the leucocytes. Without being unduly conservative the most that one can say is that the occasional normoblasts and the occasional punctate basopilic cells, as well as the eosinophile myelocytes and other young forms of eosinophiles are slightly suggestive of immaturity or youthfulness of the elements of the blood concerned. Even if the presence of immaturity is assumed, it would be necessary to establish if this is something inherent in the porphyrin animal or perhaps merely a compensatory reactive process, before definitely regarding it as evidence which favours the anabolism theory of porphyrin genesis. In a porphyrin animal exposed to the sun, secondary compensatory processes are probably present but the same compensatory processes have been eliminated in this animal by confining him to a dark stable. If the porphyrins can destroy red cells even in cases where the porphyrin animal has been adequately protected against the harmful rays of the sun, as is implied by the statement of Borst ond Königdroffer that " Die Porphyrine wirken schädigend auf die roten Blutkörperchen ", can probably only be definitely established when specimens from an animal which has been kept in a dark stable for a considerable length of time are available for pathological examination. The blood picture (5·5 milloin cells) and the marked general improvement in condition, etc., of animal 7018 in the dark stable, suggest that porphyrins do not produce markedly harmful effects on the red cells under such conditions, but previous to that, when the animal was exposed to the sun, such a destruction of the red cells possibly did take place. Except for the slight suggestion of immaturity of its blood picture, animal 7018 is in good health and daily putting on weight in the dark stable. Porphyrin excretion in the urine continues. The urine is no longer as dark in colour as it was whilst the animal was exposed to the sun, and it seems likely that a good leal of the brown discolouration of the urine may be due to the effects of photosensitization. If the porphyrins are due to the persistence of a foetal process, this would not seem to produce any disturbance of haemoglobin metabolism, in the absence of sunlight. If there is only one kind of haemoglobin, it is difficult to understand why a portion of the building stones of that kind of haemoglobin should be picked out for a maturation arrest and the remainder allowed to proceed to full maturation. If the dualism of haemoglobin postulated by Fischer were proved this would not be so difficult to accept, as the inherited deficiency may then be present only in the one haemoglobin, and the other remains unaffected. Such a maturation arrest at the porphyrin stage of haemoglobin synthesis, would bring the anomaly into line with the maturation arrest of the white cells postulated by Fitz-Hugh and Krumbhaar (1932) (Paper not available) quoted by Darling, Parker and Jackson (19[illegible]6) in Agranulocytosis, as well as with the erythroblast arrest in pernicious anaemia, also quoted by Darling and co-workers.

It is of course possible that in the absence of the effects of photosensitization red cell destruction takes place, but not at the same rate as when the animal is exposed to the sun; consequently young forms of red cells as well as leucocytes may be present in the blood, as the result of compensatory hyper-activity of the haemopoietic tissues. This compensatory process in the absence of exposure to the sun may be able to maintain the elements of the blood at a more or less normal level. No confirmatory evidence of this could be established clinically in animal No. 7018. The serum of this animal is yellow, this is due to the presence of lipochromes, but bile pigments are completely absent.

The pigments present in the two animals which were killed, seem on the whole to corespond with those described by Borst and Königsdorffer in the case of Petry. There is perhaps less iron in these animal cases. These animals were almost daily exposed to the sun and one assumes that this was not the case with Petry. In the circumstances it would seem that the effects of photosensitization are not mainly responsible for the presence of iron in the various organs of porphyrinuria cases. It is, however, almost certain that the protective mechanism of bovines against photosensitization in virtue of the hair coat and pigmented skin is much more efficient than in the case of the human subject and consequently one cannot on the above ground exclude the possibility that the effects of photosensitization may be responsible for the iron in the organs of porphyrinuria cases, even though a smaller amount of such iron is present in animal cases, in which a more intensive exposure to the harmful rays of the sun took place. In addition to prophyrinuria, Petry also suffered from other complaints. One of these two animals was definitely (and the other one probably also) infected with bilharzia. Therefore in neither instance is one dealing with an uncomplicated porphyria case and until such a case, in which the effects of photosensitization have in addition also been controlled, is available for biochemical and pathological examination, one will be at a disadvantage in interpreting some of the pathological findings.

Nomenclature.—There seems to be no sound reason why in the present state of our knowledge the nomenclature of this condition in animals should not be brought into line with the more correct terminology used in medical literature. Porphyria would seem to be the most suitable designation for the general state of the condition: porphyrinuria, where porphyrins are excerted in abnormal amounts in the urine, and porphyrinaemia for the abnormal presence of porphyrins in the blood plasma. Pink tooth is suggested as a popular name for animal cases, thus emphasising the clinical feature of the anomaly by means of which it can be easily differentiated from redwater (piroplasmosis).

SUMMARY.

1. The occurrence of congenital porphyrinuria in 13 bovines, all of which are the progeny of one bull, is described.

2. 77 per cent. of the affected animals are males and 23 per cent. are females. In this respect the incidence of the condition resembles the analogous condition in humans as well as the incidence of recorded cases of Alcaptonuria and albinism, thought to be hereditarily transmitted as recessive characters.

3. The available evidence strongly suggests the hereditary transmission of the condition through a particular bull.

4. Details of the clinical symptoms of four cases are presented. These are the first animal cases of the anomaly available for clinical examination. The teeth are brownish pink, but the enamel is not pigmented. The poor condition and the scabs and crusts on parts of the skin unprotected by hair, are mainly due to the effects of photosensitization. The urine is amber, brown or reddish brown in colour and contains amongst other uro- and copro-porphyrin.

5. " Pink tooth " is suggested as the popular name for the animal cases. This emphasises the clinical feature of the anomaly by means of which it can be easily differentiated from redwater (piroplasmosis).

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Some Cases of Congenital Porphyrinuria in Cattle: Chemical Studies upon the Living Animals and Post-Mortem Material.

By CLAUDE RIMINGTON, Section of Chemistry, Onderstepoort.

(Received for publication February 13th, 1936.)

SEVERAL living bovine cases of chronic Porphyry or congenital Porphyrinuria were recently encountered on a farm near Bremersdorp in Swaziland. An account of the discovery of these cases and of the clicinal picture they present is being submitted by Fourie (1936). The condition, which also occurs in human beings is extremely rare and for this reason the opportunity of working with living animal cases is to be considered very fortunate. Fourie has reviewed existing knowledge of the disease from its clinical aspect and has also stressed the difference between true porphyry and "ochronosis" where the pigment deposited in the bones appears to have the character of a melanin. Congenital porphyrinuria would seem to be an inherited metabolic anomaly comparable in some respects with alcaptonuria (Garrod, 1923); however, the meagre data available in records from human and veterinary medicine is insufficient to do more than lend a suggestion of the hereditary factor in the transmission of the disease. The present cases include both males and females, all the progeny of a single pure-bred shorthorn bull. This bull, together with a few normal heifers he has sired and normal cows which have produced affected calves, is at present at Onderstepoort and will be used for breeding purposes.* Controls will be drawn from the Laboratory herd.

Since Fourie intends to discuss the disease from a general standpoint, it is proposed to confine this article to the strictly chemical aspect and to record the chemical examination of one case which was slaughtered for the purpose of investigation. Blood, urine, faeces, bile, bones, bone-marrow, liver, spleen and kidneys were examined. The pathology will be considered in a later paper by one of the collaborators in this investigation.

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* On examination, the animals were found to be suffering from bilharziosis. They are being treated for this condition before accurate clinical studies are made. Bilharzia urines which I have examined have, however, contained no more than the usual trace of coproporhyrin (see Appendix).

Of the 15 or so human cases of porphyry which have been noted (compare Günther 1925) only one, that of the man Petry, has been studied in any detail and, with the exception of an isolated observation, that an ox prior to slaughter " was supposed to have passed blood-stained urine " (Schenk 1902) no record of a living animal case exists. Upon Petry's death, his bones, organs, blood, bile, etc., were examined chemically by Fischer, Hilmer, Lindner and Pützer (1925) and the histo-pathological examination carried out by Borst and Königsdörfer (1929) who utilised for this purpose the technique of ultra-violet fluorescence microscopy and published their results in a classical monograph.

Perusal of the literature shows that a chocolate colouration of the bones of slaughtered animals has been observed by Mettam (1910), Witte 1913), Schmey (1913 a; 1913 b), and Maraev (1928). Schmey (1913, b) and Poulsen (1910) were among the first to recognise this condition as an entity distinct from " ochronosis " a term introduced originally by Virchow in 1866 with reference to human material in which the pigment was subsequently proved to be of melanin nature.

The bone pigment of animal porphyry was first identified as uroporphyrin by Fink (1931). The case was that of a bovine and the only material available was 19 gm. of rib, 23 gm. of vertebrae and 5 gm. of bone marrow, together with a piece of kidney. Neither marrow nor kidney yielded any result. The pathological examination of the case was carried out by Fikentscher (1931) who found some lamellae of the bones, both periosteum and endosteum, to be stained by a diffuse brown pigment exhibiting in ultra-violet light a rosy fluorescence with the following spectral bands:—

692; 670-647; 632-614; 596-575.

These figures correspond with the ultra-violet emission spectrum of uroporphyrin as determined by Borst and Königsdörfer.

Shortly afterwards, a further case was encountered in the Leipsig abattoir and was studied by Fink and Hoerburger (1931) who succeeded in crystallising the uroporphyrin ester (M.P. 293°) from the bones (15 mgm. from 630 gm. bones) and identifying it as uroporphyrin I by comparison with a specimen originally isolated from Petry urine. They employed for the comparison the elegant pH-fluorescence technique developed by Fink and Hoerburger (1933 a, 1933 b, 1934 a, 1935 a, also Hoerburger 1933).

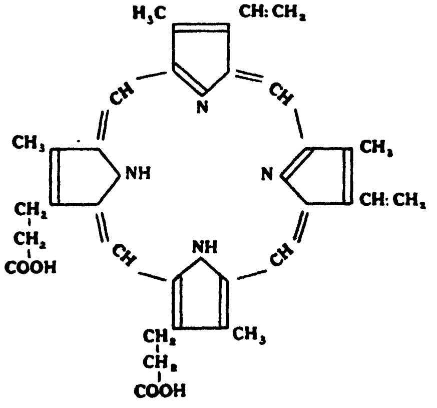
Yet another case has been reported upon during the past year (Fink and Hoerburger 1935 b) and the appearance of concentric rings of lighter and darker colour in a cross section of the bone described. This feature had not previously been encountered by Fink and Hoerburger but it may be stated at once that in skeletal material from both the cases which have been studied during the course of the present work, similar annular zones of pigment were very clearly defined. They most probably represent accumulations of pigment deposits during periods of arrested bone growth rather than periodic variations in the intensity of the disease.

Up to the time that the present studies were undertaken, no examination of the urine, faeces or organs of affected animals had been made nor was any report upon the clinical condition available.

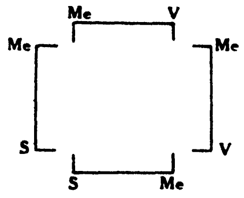
THE NATURAL PORPHYRINS.

In order to illustrate the following discussion of the experimental findings, it is felt advisable to present a brief summary of the chemistry of the porphyrins, their configurational and isomeric relationships and the present state of knowledge concerning their occurrence in nature [for general reviews see Fischer (1931), Kämmerer (1933)].

By removal of iron from haematin, is obtained the substance protoporphyrin (Fig. 1), a complex of four pyrrole nuclei united by methine bridges -CH= and possessing, as substituents, four methyl groups CH_3, two vinyl groups $-CH=CH_2$ and two propionic acid groups; $-CH_2 \cdot CH_2 \cdot COOH$, in the positions shown. A convenient shorthand method for writing such formulae has been introduced by Fischer in which only the pyrrole rings are represented and these merely by the sign X┌────┐Y the substituents X and Y being in the $\beta\beta'$ positions. A reference to the example of protoporphyrin will make this clear (see Fig. 2).



Protoporphyrin III



Protoporphyrin (abbreviated)

Figs. 1 and 2.—Protoporphyrin III

Key:

$Me = CH_3$.
$V = CH=CH_2$.
$S = CH_2 \cdot CH_2 \cdot COOH$.

Inspection of the formula for protoporphyrin will show that numerous isomeric modifications are possible depending upon the way in which the different substituent groups are arranged. This number becomes reduced to four in the case of aetioporphyrin, a substance obtainable from protoporphyrin, in which the substituent groups are 4 ethyl and 4 methyl groups only. The four aetioporphyrins, aetioporphyrin I, II, III, and IV (see Fig 3) can thus be regarded as the starting points of four series of derivatives. Actually, those belonging to the series II and IV have so far not been encountered in nature so that they can, for all practical purposes be dropped from this discussion. There remain the members of the I and III series.

H. Fischer and his collaborators have shown in a brilliant series of researches, culminating with the synthesis of haematin (Fischer and Zeile 1929; Fischer 1929) that both haemoglobin and chlorophyll belong to series III, whereas the porphyrins encountered in disease frequently belong to series I.

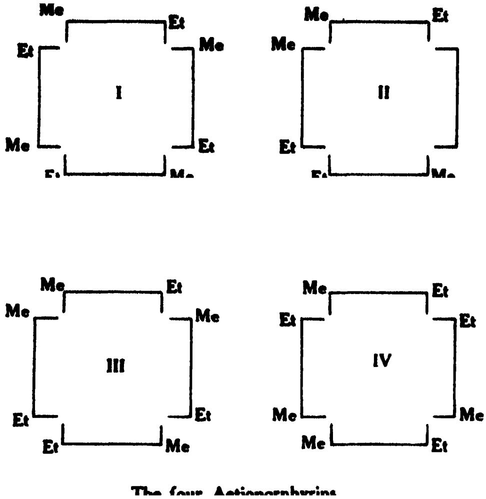
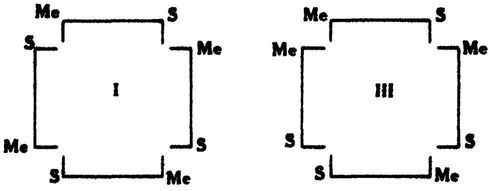


Fig. 3.—The four Aetioporphyrins.

The distinction is important, since it will be realised that the assignation of any particular pigment to the series I at once precludes its formation from normal haemoglobin by breakdown. Passage from one series to the other could only be effected by complete dissolution of the structure into its constituent pyrrole units and resynthesis in the new configuration.

Protoporphyrin, as already pointed out, possesses two carboxyl and two vinyl groups. On replacement of the latter by propionic acid residues, a tetra-carboxylic porphyrin would result. This is, in fact, the structure of the coproporphyrins (for synthesis see Fisher and Andersag 1926, and Fisher, Platz and Morgenroth, 1929), whilst uroporphyrin possesses, in all, eight carboxyl groups, arranged as can be seen by reference to Figs. 4 and 5. Since the free porphyrins do not possess sharp melting points, they are usually converted into their crystalline methyl esters for identification.



Ester M.P. 245°   Ester M.P. 169°

Coproporphyrins I and III

Fig. 4.—Coproporphyrins I and III.

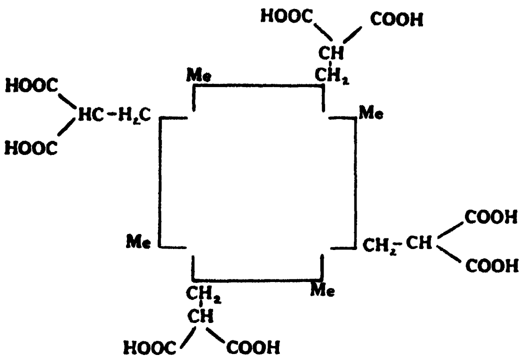


Fig. 5.—Uroporphyrin I.

Occurrence and Distribution of the Porphyrins.

The porphyrins are fairly widely distributed in nature, thus small quantities of coproporphyrin occur in normal urine and faeces (Fischer, 1915/16, Garrod, 1892, Fischer and Zerweck, 1924 a), in yeast, plants (Fischer and Hilger, 1924, a) and other materials, whilst the pigment of the tail feathers of the Turaco (*Turacus corythaix*) is the copper complex of uroporphyrin (Church, 1869; Fischer and Hilger, 1924 b) and the colouring matter of the egg-shells of many birds has been shown to be protoporphyrin [= ooporphyrin = Kammerer's porphyrin] (Fischer and Kögl, 1923; Fischer and Kögl, 1924).

Thanks to the synthetic work of Fischer and his associates, we are now in a position to assign most natural porphyrins, if isolated in sufficient quantity, to their respective isomeric series, by comparison of the ester melting points with authentic material. The importance of thus distinguishing between the haemoglobin or III series pigments and the I series can not be over-emphasised as without this knowledge, speculation as to the mode of origin or function of any pigment is groundless.

To attempt a complete survey of the occurrence of porphyrins would be too lengthy a task, consequently only the most important data will be mentioned here.

Uroporphyrin was first isolated by Fischer (1915; Fischer and Zerweck, 1924, b) from the urine of the porphyrinuric patient Petry and characterised as an octa-carboxylic acid. From the faeces of the same case a porphyrin with four carboxyl groups, coproporphyrin was isolated (Fischer, 1915/16). It occurred in smaller quantities together with uroporphyrin in the urine (Fischer, 1916, a). These two pigments have since been shown to belong to the I series, and to be present in other cases of congenital porphyry.

Even in normal urine and faeces (Schumm, 1923; Fischer and Zerweck, 1924, a, 1924, c) small quantities of coproporphyrin are known to occur.

Fink and Hoerburger (1934, b) have performed the signal service of characterising the urinary constituent as coproporphyrin I.

The normal excretion of an unphysiological coproporphyrin which can not be derived from haemoglobin breakdown, is at first sight difficult to explain. It might be thought that it was derived from disintegrated cytochrome or catalase molecules but the evidence available to date indicates that the porphyrins derivable from the latter substances belong to the III series (Stern, 1935; Zeile and Reuter, 1933). Van den Bergh, Grotepas and Revers (1932) demonstrated the existence of small quantities of protoporphyrin in the red blood cells of healthy human subjects [confirmed by Kämmerer (1933 and Schreus (1934)] and were also able to show that added protoporphyrin, in liver perfusion experiments, was converted, in part, into coproporphyrin, eliminated via the bile. They were clearly of the opinion at the time that the protoporphyrin of the red cells is protoporphyrin III and that the biliary and urinary coproporphyrins belonged to the same series. No evidence is available which would assign the protoporphyrin of the erythrocytes to either series and to the writer it seems much more probable, in view of recent developments, that it will ultimately prove to be a series I pigment. The relationship between it and the urinary porphyrin would then become clear and the Van den Bergh experiments fall into their logical place.

A clue is perhaps to be sought in the examination of foetal blood and this it is hoped to do at this Laboratory. According to Finkentscher (1935), the serum of the normal human foetus at the 4th or 5th month contains 8-10 γ per 100 c.c. of an ether-soluble porphyrin resembling coproporphyrin, the quantity slowly decreasing to 1-3 γ at the time of birth. Subsequently, as shown by Herold (1934), there is, during the first five or six days of extrauterine life, a pronounced excretion of porphyrin by the infant, possibly to be correlated with the extensive breakdown of red cells which then occurs (compare Volhard, 1930). Haurowitz (1935) has shown that there is a foetal type of haemoglobin, different in crystalline form and other properties from normal-adult haemoglobin and it seems to the writer of the greatest importance to ascertain whether the pigment moiety belongs to the I or III series. This finding in conjunction with the study of the foetal porphyrins and those of pernicious anaemia and congenital porphyrinuria might help to elucidate the whole problem of haemoglobin synthesis and metabolism and afford evidence against or in support of the oft-expressed view (compare Ehrlich, 1892; Duesberg 1931) that in the two diseases mentioned the organism exhibits an atavistic tendency so far as its pigment metabolism is concerned. Watson (1935, a) has already shown that coproporphyrin I is eliminated in the faeces of pernicious anaemia cases but disappears following liver therapy.

Certain states of intoxication in the adult are followed by excretion of porphyrin and the evidence is gradually accumulating to show that the pigments, in this instance, frequently, although not invariably belong to the III or normal haemoglobin series. Thus, Grotepass (1932) identified the coproporphyrin present in the urine after lead poisoning as belonging to the III series (see also Hoerburger and Fink, 1935) whilst Fischer and Duesberg (1932) have confirmed this finding in experimentally produced lead poisoning cases. Schreus (1935) and Hoerburger and Fink (1935), report that coproporphyrin III is present in the urine after the administration of salvarsan. Injury to the liver or interference with its excretory function, as in icterus, also leads to porphyrinuria, the pigment in this case proving to be coproporphyrin I so far as accurate investigations have been made. Watson (1935, b) identified coproporphyrin I in the urine of a case of cirrhosis of the liver occasioned by cinchophen. He (1935, c) also obtained the same pigment from the faeces in a case of familial jaundice at the haemolytic crisis and considers it to be most probably derived from the protoporphyrin of the red cells (cf. Van den Bergh, Grotepass and Revers, 1932).

As already stated, uroporphyrin has been isolated from the urines of several human cases of congenital porphyrinuria and in most instances the pigment was assigned to the I series. Very marked irregularities in the melting points of the octa-methyl esters have, however, occurred and this circumstance has led Fisher and others to suspect that mixtures of isomeric uroporphyrins might be present. Such isomers can hardly be position isomers since in some cases at least (Fischer and Duesberg, 1932) the uroporphyrin has been converted chemically into the corresponding coproporphyrin which proved to be identical with coproporphyrin I. A still further complication arises on account of the fact that two distinct types of porphyrinuria are recognisable, the chronic form ("Haematoporphyria congenita") as typically exemplified in the case of Petry, and an acute form, "acute idiopathic porphyry" (Haematoporphyria acuta idiopathica), which presents certain characteristic features. Thus, acute idiopathic porphyry usually makes its appearance at a comparatively late stage in life, after adolescence; the attacks are periodic, and the excretion of porphyrin in the urine paroxysmal. Attacks are accompanied by severe colic, nervous symptoms and even muscular paralysis but pronounced photosensitivity, the most noticeable symptom in true congenital porphyrinuria, has never been recorded as an accompaniment to the acute form. Some tendency towards this condition is probably to be inferred, however, from the brown pigmentation of exposed skin surfaces and even blistering reported in the cases of Günther (1922), Brown and Williams (1909), and Eichler (1932).

These differences are well brought out in the review by Günther (1925) and the chemical aspect is now receiving attention from Waldenström, Fink and Hoerburger (1935). The melting points of the octa-methyl esters of the urine porphyrin found by different workers, both in cases of acute and of chronic porphyrinuria, may be summarised as follows. Cases of porphyrinuria due to poisoning by lead, trional, sulphonal, etc., are omitted.

Acute porphyrinuria.

Loeffler (1919)	**262°**	—
Weiss (1925)	**274°**	**Faeces contained coproporphyrin I.**
Fischer and Duesberg (1932)	**269°**	**Decarboxylated to coproporphyrin I.**
Waldenström, Fink, and Hoerburger (1935)	**243°**	**Separated into fractions easily soluble in ethyl acetate (238°) and sparingly soluble (258°).**

Chronic porphyrinuria.

Fischer (1915)	293°	Petry case. Urine.
Fischer and Zerweck (1924, b)	286–7°	Petry case. Urine. Other fractions with lower M.P. prepared.
Fischer, Hilmer, Lindner, and Pützer (1925)	285°	Petry case. Bones.
Mackey and Garrod (1925–6)	283°	Case G.L. Faeces corproporphyrin I (248°).
Jost (1927)	273°	—
Fischer and Zerweck (1924, c)	275°	Molzberger case. Raised only to 279° by saponification and re-esterification.
Van den Berg, Regniers, and Muller (1928) Van den Bergh, Muller, and Hijman (1929)	Copro, 160–1°	Coproporphyrin III. Case known as "Van den Bergh's case".
Fischer, Platz, and Morgenroth (1929)	Above specimen purified, 169°	
Fischer and Duesberg (1932)	Copro only 144–8° Remelt, 160–8°	Case K. Coproporphyrin III only.
Fischer and Duesberg (1932)	281°	Case L.

It will be seen that only two cases of chronic porphyrinuria have so far occurred in which the pigment (coproporphyrin) excreted proved to belong to the III series. In all other instances the evidence suggests that the pigments belong to the I series.

Mention must be made of the case recorded recently by Van den Bergh and Grotepass (1933) of a man who for 20 years had suffered from a porphyrinaemia of gradually increasing intensity without, however, excreting more than the normal trace of porphyrin in his urine. Coproporphyrin I was isolated from the faeces but uroporphyrin could nowhere be found. The patient suffered from a fairly severe nephritis, his urine containing much albumin and one is led to suspect that the damage to the kidney was responsible for the failure to excrete an increased quantity of coproporphyrin. It might also be assumed, however, that the transformation of coproporphyrin to uroporphyrin normally took place in the kidney, a view for which there is some evidence (see Discussion later) and that in the diseased state of the organ in this particular case, such power had been lost. The site of formation of uroporphyrin is still not known with certainty. In true congenital porphyrinuria it would appear that some uroporphyrin, at least, arises in the bone marrow but in the acute form of the disease, this may not be the case. To the writer, it seems essential that acute and chronic porphyrinuria should be recognised as two different diseases, having a differing aetiology, and confusion avoided when speculating upon the course of the pigment metabolism in each.

CHEMICAL EXAMINATION OF THE SWAZILAND BOVINE CASE.

The case was that of a young reddish-brown castrated male (2 years 4 months old). The animal was in poor condition and showed lesions round the eyes and muzzle resembling those caused by photosensitisation. In addition, in the centre of the back, just where the hair fell away, there was an oval-shaped sore measuring 6 inches by 3 inches. The skin round this lesion was hard and keratinous (see Figs. 6 and 7). The appearance was consistent with the suggestion that the animal was photosensitive, but protected, over the greater part of its body, by the coarse reddish-brown hair of the coat.

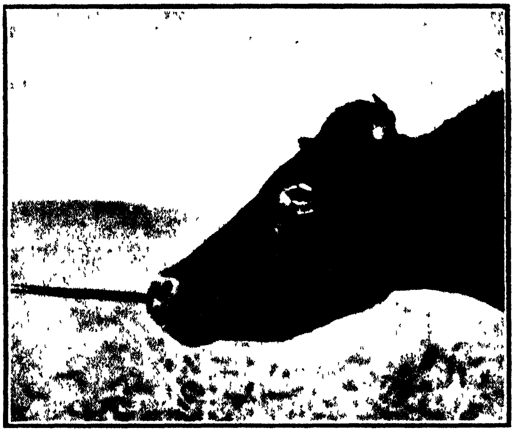


Fig. 6.—Bovine case of congenital porphyrinuria showing lesions due to photosensitisation.

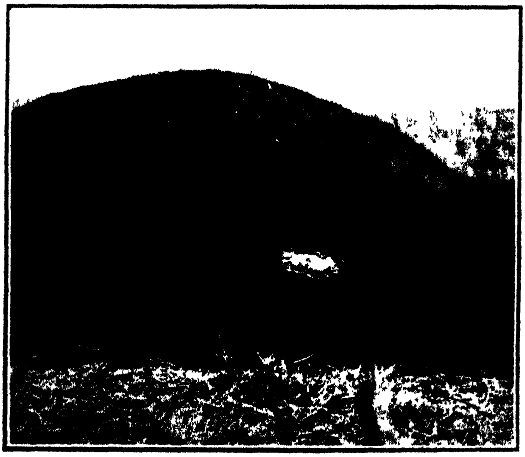


Fig. 7.—Same case after slaughter photographed to show keratinised epidermal sore on back.

Whilst under examination, it passed urine, a specimen of which was coloured a deep port-wine red colour and exhibited the following absorption bands (centres).* Fischer's (1916 b) figures for Petry's urine are given for comparison.

	614·0; 580-560; 540; 500.
Petry urine	614·5; 567·5; 535·5; 517.

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* All spectroscopic measurements made with a Zeiss grating hand spectroscope. Centres of absorption bands quoted in mμ.

The colour of the urine was seen to deepen on exposure to light.

The animal was anaesthetised by an injection of chloral hydrate and bled to death, the blood being collected with aseptic precautions in sterile evacuated flasks containing anticoagulant. In all, about five litres was obtained. The carcass was then cut open and the entire liver, spleen and kidneys removed as carefully as possible and wrapped in formalin-soaked muslin cloths. Specimens were collected in formalin and in alcohol for histological examination. The gall bladder was emptied into a sterile bottle, yielding about 300 c.c. of clear, dark-green bile. The entire skeleton was removed, cleaned from adhering muscle and wrapped in formalin cloths. It was noted that all calcified parts were deep chocolate brown in colour, whilst the cartilages and periosteum presented a normal appearance. A specimen of faeces was also collected from the large colon.

These materials were brought back to the Laboratory immediately and placed in cold storage. There was, in no instance, any indication of decomposition or putrefaction. The chemical examination was then conducted at leisure, taking one organ at a time and employing, as a general rule, the acetic acid-ether method introduced by Fischer followed by extraction of the residue with dilute ammonia to remove any uroporphyrin. Ammonia was found more convenient than pyridine and yielded excellent results.

Urine.

The bulk of the urine, which was free from albumin, was acidified with acetic acid and left in the ice-chest for several days until the precipitate had flocculated out and could be centrifuged off. The supernatant was still dark brown in colour but only exhibited a very faint porphyrin spectrum, together with a more marked broad band with centre 495·5 mμ. The precipitated porphyrin was esterified by methyl alcoholic hydrochloric acid, and the ester transfered to chloroform from which it was crystallised in the usual way. The crude uroporphyrin ester was washed repeatedly with boiling methyl alcohol, thereby eliminating a quantity of brown pigment possessing no absorption spectrum, and finally recrystallised several times. It had the usual appearance of uroporphyrin octa-methyl ester (see Fig. 8) and M.P. 275-7°.* The absorption spectrum in chloroform was as follows:—

626·1; 580·9; 570·8; 535·2; 500·7.
faint

Uroporphyrin methyl ester*:

626·1; 581·4; 570·5; 536·0; 500·8.

A specimen of the crude free porphyrin, dissolved in N/10 NaOH exhibited the following spectrum:—

614; 539·3; 502·8.

Uroporphyrin has 612; 539·0; 503·7.

It flocculated rather slowly on the addition of acetic acid.

---

* Melting points observed on the electrically-heated Kofler micro-melting point apparatus.

* Absorption spectra data taken from "Tabulae Biological", Vol. 3, or if not there recorded, from the original sources.

It was noticed that the mother liquors of the first one or two ester recrystallisations contained considerable quantities of pigment. When examined in a thick layer, the following absorption bands were seen to be present:—

642; 622·4; 602·2; 566·5; 531·8; 499·2.
faint

With the exception of the band at 642, these figures suggest a coproporphyrin. The solution was accordingly concentrated and left in the ice chest when a crystalline deposit formed consisting of a mixture of uroporphyrin ester and short stoutish prisms with slightly oblique ends. A partial separation was effected by repeated recrystallisation, the prisms melting ultimately at 233-5° and giving in chloroform the following spectrum, from which it was concluded that the substance was in all probability coproporphyrin I. (Compare however Fischer and Zerweck, 1924, b.)

623·6; 597·9; 567·3; 533·7; 498·6.

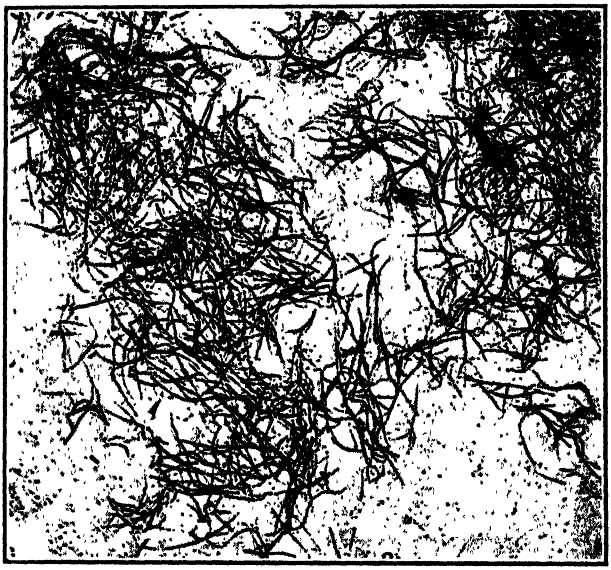

Fig. 8.—Uroporphyrin ester, M.P. 277°, from urine. × 245.

Coproporphyrin I was also isolated directly from a further quantity of the original urine by the acetic acid-ether technique. The ester in chloroform (M.P. 235°) had the spectrum:—

622·4; 596·5; 577·1; 566·8; 530·6; 497·7.

Coproporphyrin has

623·9; 597·3; 577·7; 568·2; 529·8; 497·9.

Transferred to 25 per cent. HCl it had:—

595·2; 575·4; 551·4.

Coproporphyrin has

593·9; 574·6; 550·9.

On shaking with chloroform, no pigment passed into the lower phase. Protoporphyrin was therefore absent.

Faeces.

The sample was extracted by the acetic acid-ether method and the pigment transferred to 2 per cent. hydrochloric acid. This solution was saken with chloroform, diluted ten times and again shaken, but no detectable quantity of deuteroporphyrin was extracted. The total porphyrin was therefore transferred to ether and from this to 10 per cent. sodium hydroxide solution. Only a very small precipitate of insoluble sodium salt separated during the course of the night. After filtration, the pigment was precipitated by neutralisation washed, dried and esterified. The ester crystallised in needle-like prisms M.P. 243-4° and exhibited the typical coproporphyrin spectrum

623·7; 597·7; 578·3; 568·6; 533·1; 497·3.

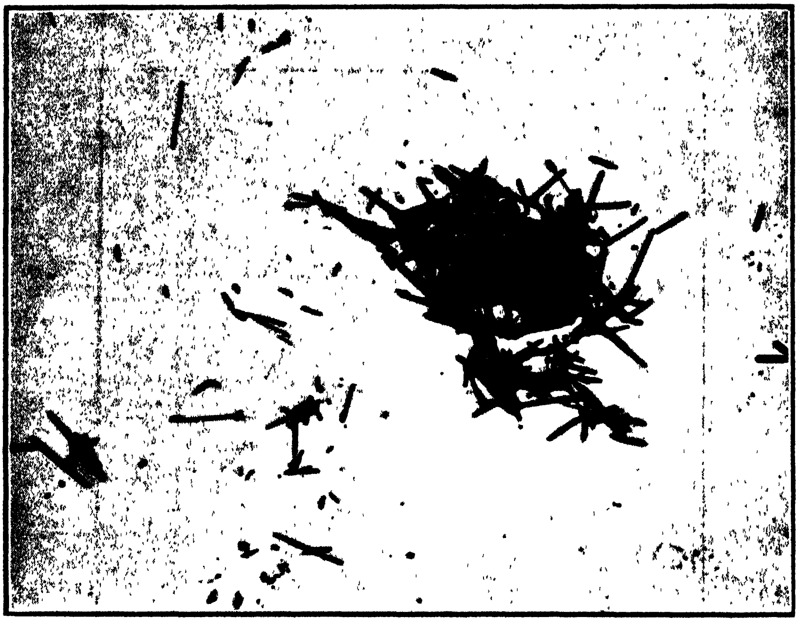

Fig. 9.—Coproporphyrin I ester, M.P. 243-4° from faeces. × 120.

It was therefore coproporphyrin I. (see Fig. 9). The mother liquor from the first crystallisation contained another much more soluble pigment with the following absorption spectrum with relative intensities of the bands as shown. It was not identified.

$$603{\cdot}4;\ \underbrace{574{\cdot}9\text{-}551{\cdot}3}_{562{\cdot}1\ \text{max.}};\ 529{\cdot}0;\ 499{\cdot}0.$$

III I II IV

As would be expected, there were present, in the original ether extract, porphyrins which did not pass into 2 per cent. HCl, most probably pigments derived from the breakdown of chlorophyll.

After removing these by shaking with 25 per cent. HCl, the ether was still rose coloured and exhibited the two-band spectrum of uroporphyrin copper complex:—

$$562{\cdot}0;\quad 526{\cdot}5.$$

Cu uroporphyrin has $562{\cdot}3;\quad 528{\cdot}2.$

Blood.

The blood, 5 litres in all, was centrifuged and the red cell precipitate washed with isotonic saline. The plasma and cells were then worked up in the usual way.

(*a*) *Plasma.*

About 3·5 litres of yellowish plasma was obtained. It gave a very faintly positive direct Van den Bergh reaction.

The 5 per cent. hydrochloric acid shakings (about 1 litre in volume), had a purplish-blue colour with a strong reddish fluorescence. The absorption spectrum corresponded with that of a coproporphyrin. The residual ether contained no copper salt.

$$595{\cdot}2;\quad 575{\cdot}4;\quad \underbrace{559{\cdot}3\text{-}543{\cdot}4}_{551{\cdot}4}.$$

Coproporphyrin has

$$593{\cdot}9;\quad 574{\cdot}6;\quad \underbrace{558{\cdot}5\text{-}543{\cdot}4}_{550{\cdot}9}.$$

The pigment was transferred to ether and washed well.
Spectrum in ether:—

$$625{\cdot}4;\quad (580{\cdot}6);\quad 568{\cdot}8;\quad 529{\cdot}3;\quad \underbrace{507{\cdot}6\text{-}485{\cdot}2}_{496{\cdot}4}.$$

Coproporphyrin has

$$623{\cdot}9;\quad 577{\cdot}7;\quad 568{\cdot}2;\quad 529{\cdot}3;\quad \underbrace{505{\cdot}6\text{-}490{\cdot}3}_{497{\cdot}9}.$$

On evaporation of the ether, a crystalline residue remained, stellate clusters of fine needle-like prisms. After washing with ether, the residue was esterified and the ester crystallised in slender needle-like prisms, M.P. 243-4° (see Fig. 10). The spectrum in chloroform confirmed the identification as coproporphyrin I.

$$623{\cdot}0;\quad (599{\cdot}2);\quad 577{\cdot}5;\quad 568{\cdot}1;\quad 532{\cdot}4;\quad 498{\cdot}9.$$

Coproporphyrin ester has

$$622{\cdot}3;\quad 596{\cdot}7;\quad 578{\cdot}1;\quad 567{\cdot}7;\quad 532{\cdot}9;\quad 499{\cdot}1.$$

The finding of coproporphyrin I in the blood plasma is of great importance since it offers an explanation of the photosensitisation from which the animal suffered, and brings the case into line with that of the man Petry and other chronic congenital porphyrinurics.

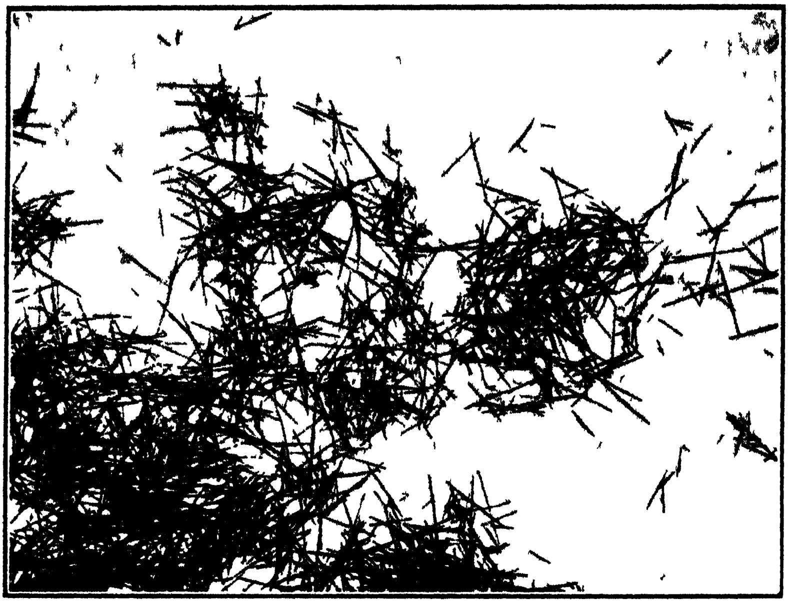

Fig. 10.—Coproporphyrin 1 ester, M P 243-4° from blood plasma × 120

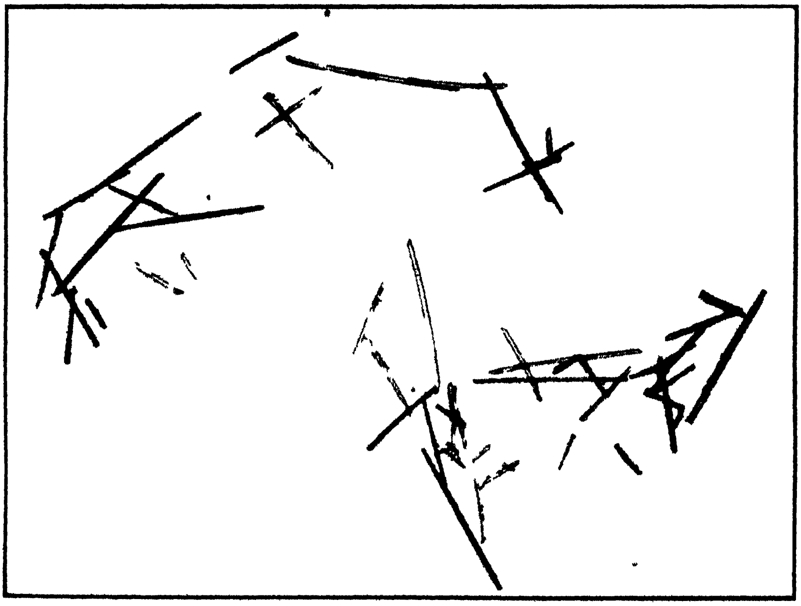

Fig. 11.—Coproporphyrin 1 ester, M.P. 241° from red blood cells. × 245

The serum protein coagulum was extracted with pyridine with the intention of seeking uroporphyrin in the extract but by an unfortunate mischance, the pyridine extract exploded during concentration and was lost.

(*b*) *Red Cells.*

The final 5 per cent. acid extract from the erythrocytes had a greenish blue colour with a red fluorescence. On transference to ether, the following spectrum was exhibited:—

648; 624·2; 599·6; 582·5; 569·4; 530; 510.

Shaking with 25 per cent. HCl removed the porphyrins leaving some haematin behind in the ether.
The acid had

595·6; 579·5; 552·3.

On shaking with chloroform, only a trace of pigment (probably protoporphyrin) was removed. The acid spectrum now agreed fairly well with the acid spectrum of coproporphyrin:—

595·3; 576·3; 551·9.

Coproporphyrin has 593·9; 574·6; 550·9.

It was transferred to ether and washed well.

The ether solution had:—

623·6; 579·5; 567·9; 529·5; 498·0.
faint

Coproporphyrin has

623·9; 577·7; 568·2; 529·3; 497·9.

After removal of the solvent, the residue was esterified and recrystallised from chloroform. It formed slightly curved, needle-like prisms of M.P. 241° (see Fig. 11) and was therefore coproporphyrin I.

The spectrum in chloroform was as follows:—

622·3; 595·8; 577·8; 567·5; 531·4; 497·3.

Coproporphyrin ester has

622·3; 596·7; 578·1; 567·7; 532·9; 499·1.

The cell residue was shaken with dilute ammonia, the extract evaporated to dryness, esterified and the ester transferred to chloroform. A small quantity of pigment was obtained corresponding spectroscopically to uroporphyrin ester.

626·6; 570·0; 534·6; 501·1.

Uroporphyrin ester has

626·1; 570·5; 536·0; 500·8.

The identification of coproporphyrin I and of uroporphyrin in the erythrocytes is also of great significance as it suggests that some of the blood cells in circulation are definitely abnormal, containing the

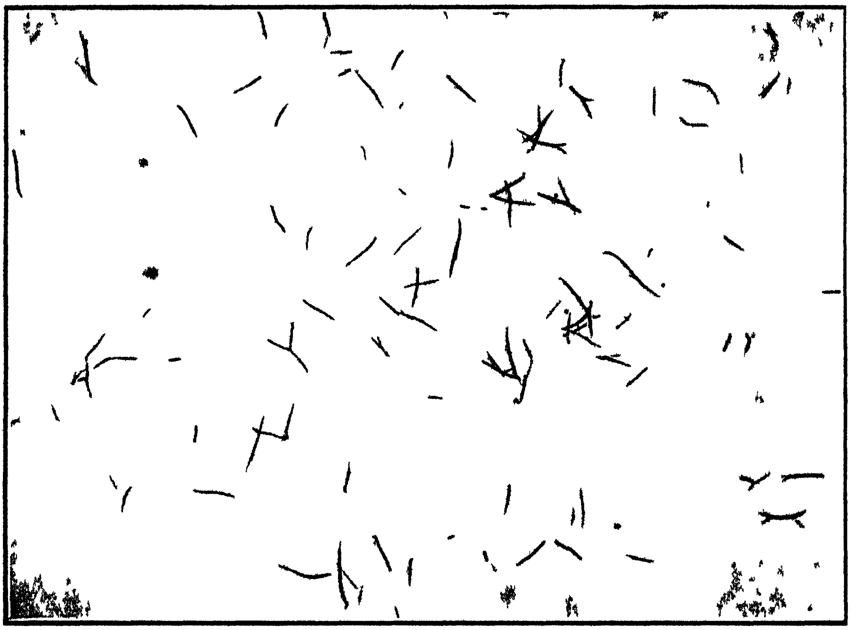

Fig 12 —Uroporphyrin ester from bones, 30 gm lot, M.P 273 4° × 250

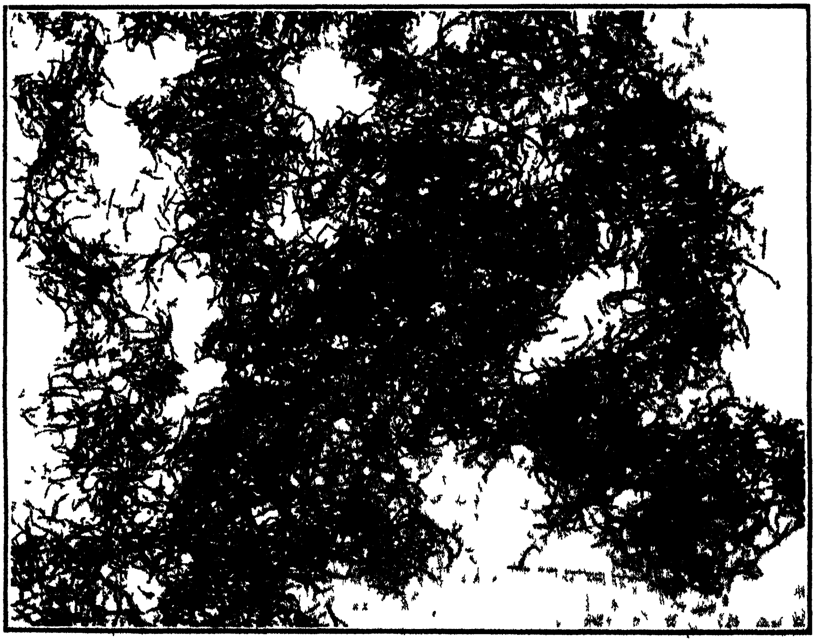

Fig 13 —Uroporphyrin ester from bones, M P. 276 7°. × 130

atypical porphyrins produced in the bone marrow. Fischer did not apparently examine the cells of Petry's blood for porphyrins although he was able to show that the haematin was of the normal type.

Bones.

Material from two cases was available, the major part of the skeleton of the steer which was slaughtered and which has been described above and a small sample (30 gm.) of bone derived from a previous case. This latter material had been preserved in formalin. Upon it, various extraction methods were tried out and the most satisfactory technique, to be described below, employed when working up the larger sample. The limb bones, which were of a dark chocolate colour, exhibited in cross section a series of concentric rings of lighter and darker staining. The cartilages were uncoloured.

The material was crushed in a bone mill and defatted by continuous alcohol and ether extraction. The dry residue was then steeped in successive changes of 5 per cent. hydrochloric acid, the last traces of pigment being removed by 20 per cent. acid, but as this solution contained much dissolved bone substance it was worked up separately. The acid extracts were evaporated to dryness in a large vacuum evaporator and the crusty residue introduced into a large volume of methyl alcohol containing 5 per cent. by volume of concentrated sulphuric acid. Sufficient of this solution was used to ensure the complete precipitation of the calcium salts. After refluxing for 4 hours, the mixture was allowed to stand overnight at room temperature and then about one-tenth of the volume of chloroform added. Ice water was then poured in until the chloroform phase carrying the pigment separated sharply. Further shakings with fresh quantities of chloroform were added to the main solution. This was washed well with water, evaporated to dryness and the residue washed repeatedly with methyl alcohol until no more brown pigment, possessing no absorption spectrum, dissolved. The residue of uroporphyrin ester was then dissolved in a small volume of chloroform, filtered and crystallised by the addition of about 5 to 7 volumes of boiling methyl alcohol. The pigment separated in the finely microcrystalline form characteristic of uroporphyrin octamethyl ester, but the yield was very small. From the skeleton of the young steer, 0·3 gm. of pure pigment was obtained. The melting point was 273-4° in the first case and 276-7° for the material from the entire skeleton and could not be raised by repeated re-crystallisation (see Figs. 12 and 13). The spectrum in chloroform corresponded to that of uroporphyrin ester:

626·0; 582·9; 571·3; 535·0; 501·2.

Uroporphyrin ester has

626·1; 581·4; 570·5; 534·7; 500·8.

A specimen of the crude extract of the bone had (in 20 per cent. HCl)

598·6; 578·1; 554·4; 463·9.

I II III IV Order III, IV, I, II.

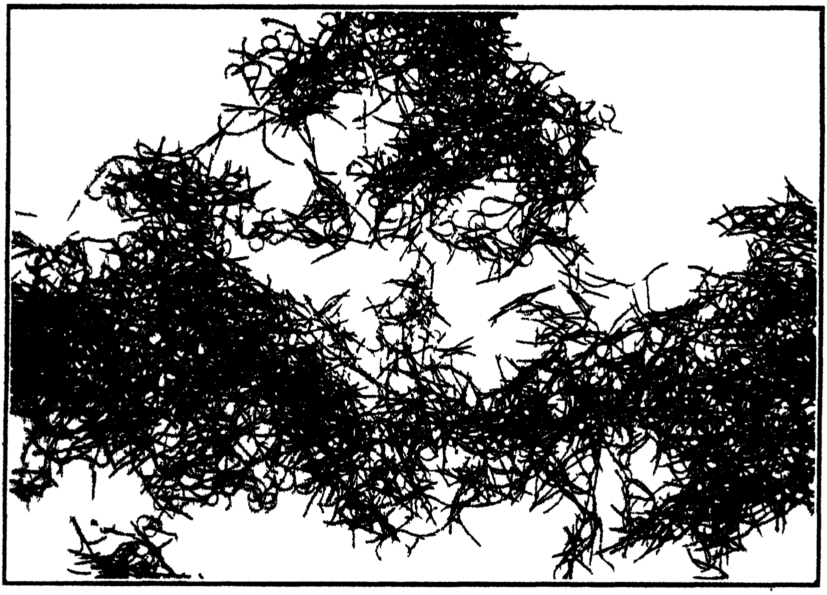

Fig 14 —Uroporphyrin ester copper complex (Bones) × 120

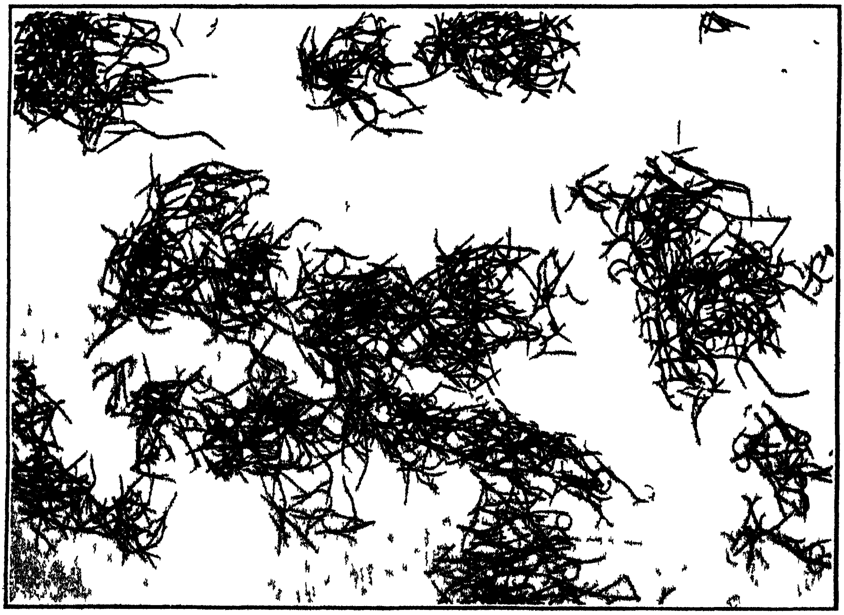

Fig 15 —Uroporphyrin ester copper complex (Bones) × 120.

Uroporphyrin in 25 per cent. HCl has

597·9; 577·6; 553·6; 511·3.

The emission spectrum in ultra-violet light was measured using a small pocket spectroscope.

665-585 with bands at 625; 600.

Uroporphyrin has

664-596 with bands (660); 624; 602·5.

*Microanalysis**:

Uroporphyrin ester $C_{48}H_{54}O_{16}N_4$

	C	H	N	CH_3O
Found	60·73	5·94	6·20	25·43
Required	61·10	5·78	5·94	26·31

The copper complex prepared in the usual way crystallised from pyridine-acetic acid in fine, red, hair-like needles M.P. 311-4° and 310-3° respectively in the two cases (see Figs. 9 and 10). Fischer gives the M.P. of the copper complex of uroporphyrin from Petry's urin as 314° but it is to be noted that in many cases where the uroporphyrin sample had a M.P. of about 275° a copper complex with lower M.P. (about 292° has been obtained. The copper salts in the present instance were repeatedly recrystallised. The absorption spectra were measured in pyridine.

Preparation I ... ... 568·8; 532·6.
Preparation II ... ... 567·4; 532·5.

Uroporphyrin ester Cu complex has 570·0; 532·5.

It was noticed, when working up the larger preparation, that the mother liquors of the first two recrystallisations contained a considerable quantity of pigment. By adding ether until precipitation occurred and recrystallising repeatedly, a fraction crystallising in fine needles (see Fig. 16) was eventually obtained having M.P. 253-5° and the following spectrum in chloroform:

626·9; 572·0; 535·8; 500·9.

These figures agree well with uroporphyrin ester. The copper complex was also prepared (see Fig. 17) and found to have the normal melting point. The occurrence of a low-melting isomer in the bulk preparation is in accordance with the experience of others for urines, notably Waldenström, Fink and Hoerburger (1935), and probably accounts for the M.P. of the main sample being stationary but lower than that (293°) found by Fischer in the first instance for uroporphyrin from Petry's urine. The matter has already been discussed on a previous page. Coproporphyrin could not be detected in the bones.

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* Microanalysis by Dr. O. Backeberg, University of the Witwatersrand, to whom my thanks are due.

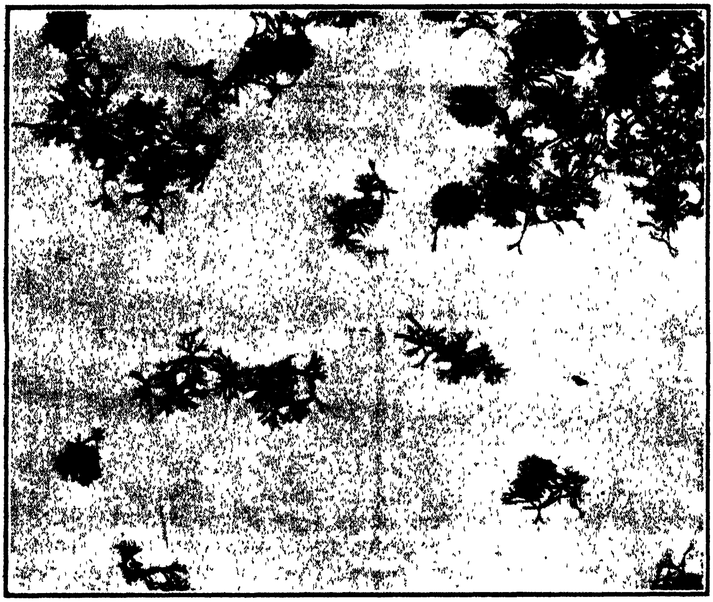


Fig. 16.—Uroporphyrin ester, M.P. 253-5°, from mother liquor of main crystallisation. × 120.

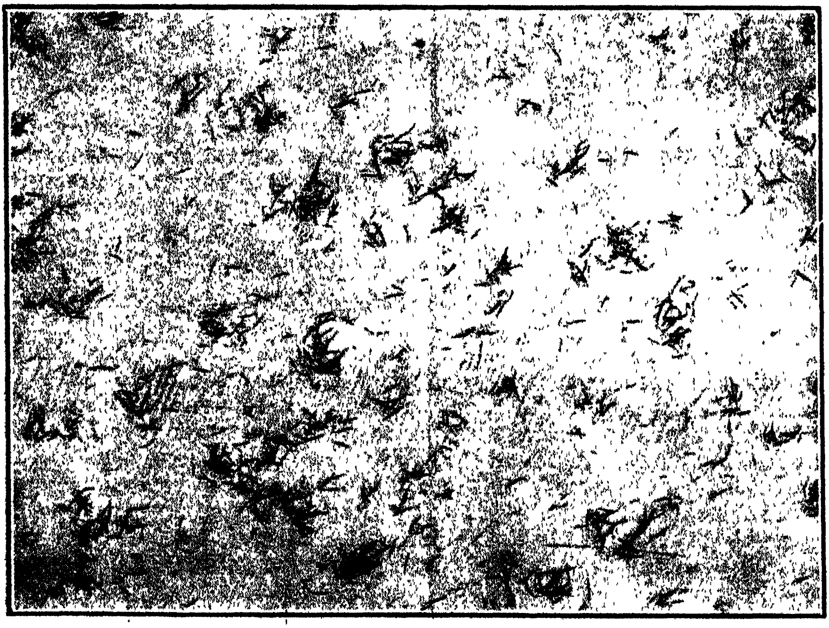


Fig. 17.—Uroporphyrin ester Copper complex from ester, M.P. 253-5°. × 120.

Bone Marrow.

This was obtained by longitudinal section of the long bones, the red marrow being removed. There was probably some admixture with pigmented bone lamellae. After defatting, the material was extracted with acetic acid-ether and this extract shaken with 5 per cent. hydrochloric acid, affording a strongly coloured acid layer exhibiting very clearly the following spectrum (the residual ether contained only haematin):

593·5; 550·9.

On transference to ether, a typical neutral coproporphyrin spectrum was obtained:—

623·0; 568·2; 528·8; 497·9.

Coproporphyrin has

623·9; 568·2; 529·8; 497·9.

The pigment was esterified and the ester, after recrystallisation, formed curved needle-like prisms M.P. 244-5° (see Fig. 18). It was therefore identified as coproporphyrin I.

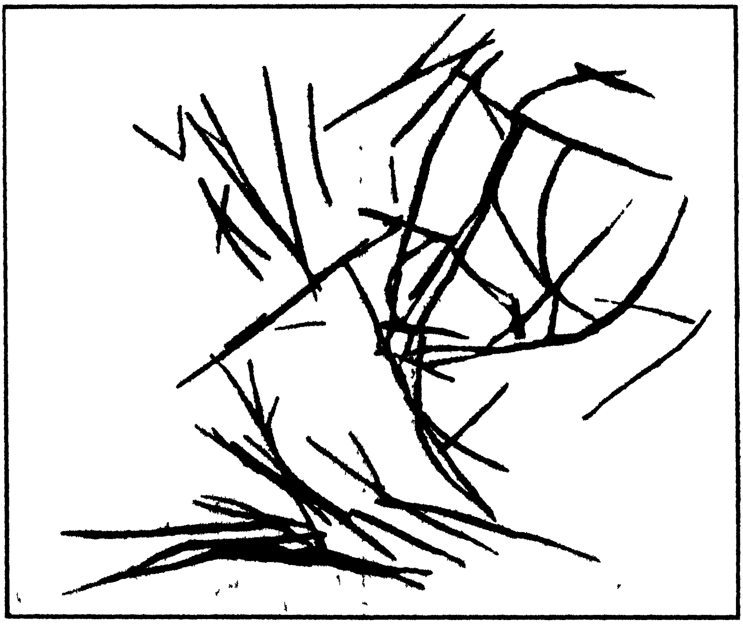


Fig. 18.—Coprophyrin I ester, M.P. 244-5°, from bone marrow. × 120.

The residue of material after extraction of the ether-soluble porphyrins was worked up for uroporphyrin in the way previously described. A good yield of ester was obtained (see Fig. 19) with M.P. 276-7° and spectrum

626·8; 581·7; 568·1; 534·2; 500·7.

The copper complex was also prepared and had in pyridine

565·0; 531·3.

The isolation of coproporphyrin I from the bone marrow and its absence from the bones is a highly significant finding, indicating as it does that the bone marrow is one, at least, of the sites where this particular pigment is synthesised

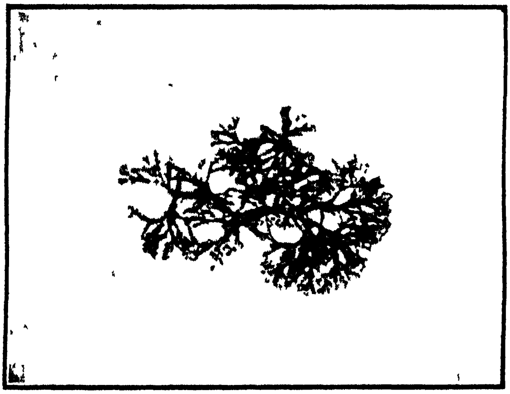


Fig. 19.—Uroporphyrin ester, M.P. 276-7°, from bone marrow. × 270.

Spleen.

The spleen was minced, steeped in glacial acetic acid and then worked up by the ether method in the usual way. The residue was subsequently extracted with a 5 volume per cent. solution of ammonia.

From the washed ethereal solution, 5 per cent. hydrochloric acid extracted a small quantity of ether-soluble porphyrin but insufficient to crystallise. The spectrum in ether corresponded to that of coproporphyrin.

624·0; 569·8; 529·5; 497·1.

Coproporphyrin has

623·9; 568·2; 529·8; 497·9.

The residual ether contained haematin. The ammoniacal extract exhibited bands at 576·5 and 541·8. It was evaporated to dryness and the residue esterified. The ester was washed with methyl alcohol and recrystallised from chloroform-methyl alcohol mixture. It had the appearance typical of uroporphyrin ester(see Fig. 20) and M.P. 278°, unchanged by repeated recrystallisation. The presence of these two porphyrins in the spleen is particularly noteworthy; uroporphyrin predominated

Liver.

The minced organ was treated exactly as described above. The ethereal solution yielded to dilute acid only a very small quantity of a porphyrin corresponding spectroscopically with protoporphyrin. Coproporphyrin could not be found although the bile (see below) was found to contain large quantities of coproporphyrin. Most probably the correct explanation of this circumstance is that the excretory function of the liver was in no way impaired and that coproporphyrin is normally eliminated together with other bile pigments via the biliary system [compare Van den Bergh, Grotepass and Revers (1932); Althausen (1931)]. Fischer and his collaborators (1925) in Petry's liver found coproporphyrin and uroporphyrin, together, possibly, with a trace of protoporphyrin. In fresh ox liver they were able to detect small quantities of protoporphyrin which was therefore regarded as a normal constituent.

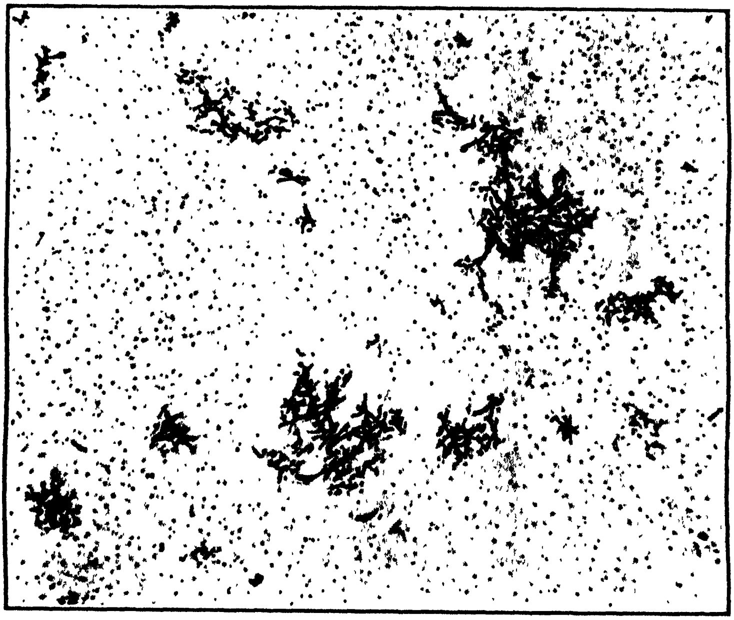


Fig. 20.—Uroporphyrin ester, M.P. 278°, from spleen. × 270.

The spectrum of the ether-soluble pigment from the present bovine case was as follows:—

(faint) 630·9; 575·6; 535·0; 500·1.

Protoporphyrin has

632·5; 575·8; 536·8; 501·9.

From the crude ester of the ammonia-soluble porphyrin, a considerable quantity of a dark brown accompanying impurity was removed by washing with methyl alcohol and the residue in chloroform showed an absorption spectrum indicating a mixture of uroporphyrin ester and its copper complex, thus:—

626·3; **566·0**; **530·0**; 501·1.

very intense

The entire material was therefore transformed into the copper salt which crystallised in fine, red needles, M.P. 313°, and exhibited in pyridine the characteristic two band spectrum:—

570·0; 532·8.

Uroporphyrin Cu salt has

570·0; 532·5.

Uroporphyrin and its copper complex would thus appear to be the only abnormal porphyrins present in the liver of this case.

Bile.

About 150 c.c. of the clear, dark green bile was used. This was mixed with acetic acid and extracted repeatedly with ether after which the residue was evaporated to dryness upon the water bath. The ether extract, after washing well with water, was shaken with 2 per cent. hydrochloric acid affording a deep purple-coloured lower phase. Ten per cent. acid, subsequently, removed only a small quantity of phylloerythrin. The 2 per cent. extract was shaken with chloroform until no more greenish-blue pigment came out (mesobiliviolin, etc.) and again shaken with fresh chloroform after dilution to 0·2 per cent. acid concentration. No mesoporphyrin or other pigment [compare Watson (1935, c) who isolated a previously undescribed porphyrin, ester M.P. 202-3°, from this fraction of the faeces in a case of familial haemolytic jaundice] left the aqueous phase. The entire porphyrin was therefore transferred to ether, affording a deep red solution with a very strong coproporphyrin absorption spectrum:—

623·9; 597·9; 577·4; 568·2; 529·3; 496·1.

Coproporphyrin has

623·9; 597·3; 577·7; 568·2; 529·3; 497·9.

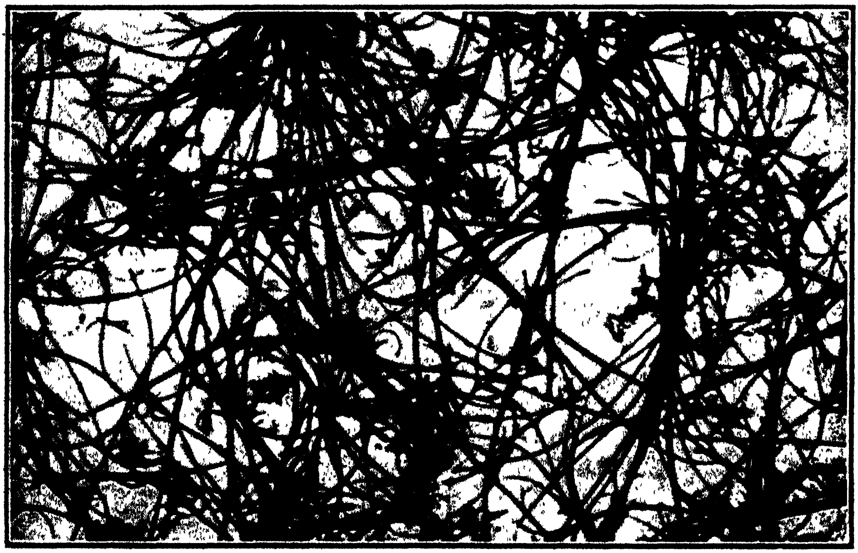


Fig. 21.—Coproporphyrin ester from bile, M.P. 237°. × 225.

The ester was prepared (6·6 mgm.) and crystallised in the long curved needles characteristic of coprophyrin I (see Fig. 21). The M.P. was 237° and could not be raised by repeated recrystallisation. It was noticed that the mother liquors of the first crystallisation exhibited a faint band in the region of 645 indicating the presence of small quantities of another pigment, probably protoporphyrin, which on standing in solution gives rise to such a band. Fischer et al. noticed a weak absorption band at 645·7 in the ether-soluble porphyrin fraction of Petry's bile.

No uroporphyrin could be detected in the evaporated bile residue after esterification, etc., by the usual methods.

Kidney.

The kidneys were somewhat dark in colour. They were minced and worked up exactly as described in the case of the liver and spleen. The 5 per cent. hydrochloric acid extract of the ether solution showed a well defined coproporphyrin spectrum but the quantity of pigment was too small to allow of crystallisation. Transferred to ether it had:—

623·9; 569·6; 529·0; 497·8.

Coproporphyrin has

623·9; 568·2; 529·3; 497·9.

Similarly, in the alkali-soluble fraction, uroporphyrin could be detected after esterification.

In chloroform it had:

628·1; 570·0; 535·5; 501·0.

Uroporphyrin ester has

626·1; 570·5; 534·7; 500·8.

In order to summarise clearly these results a chart is presented below indicating the pigments, with their ester melting points, isolated from the various organs. A similar chart showing the findings in Petry's case has been prepared, for comparison, from the published data of Fischer, Hilmer, Lindner and Pützer (1925). Where pigments were isolated in crystalline form and their identity confirmed by melting point determination, the entry is made in bold type, but where spectroscopic data alone was relied upon, italic type has been used.

CHART I.

Experimental Findings in Bovine Case of Congenital Porphyrinuria

Urine	**Uroporphyrin I, 275–7°**	**Coproporphyrin I, 233–5°**	**Some urobilin and brown pigment without spectrum.**
Faeces	***Uroporphyrin Cu Salt*** **(spectroscopically)**	**Coproporphyrin I, 243–4°**	***Unidentified pigment*** **with absorption spectrum 603; 562; 529; 499.**
Blood plasma.	—	**Coproporphyrin I 243–4°**	—
Blood cells	***Uroporphyrin*** **(in traces, spectroscopically)**	**Coproporphyrin I 241°**	—

CHART I—(*continued*).

Bones	Uroporphyrin I Case i 273–4° Case ii 276–7° (synthetic Cu salts 311–4° and 310–3° respectively)	Nil.	From mother liquors a more soluble uroporphyrin fraction 253–5°
Bone marrow	Uroporphyrin I, 276–7°	Coproporphyrin I, 244–5°	
Spleen	Uroporphyrin I, 278°	*Coproporphyrin* (spectroscopically)	
Liver	Uroporphyrin I and Cu complex 313°	Nil.	*Protoporphyrin* (spectroscopically; normal constituent). Much dark brown pigment spectroscopically negative.
Bile	Nil.	Coproporphyrin I, 237°, relatively large quantity	Possible traces of products derived from Protoporphyrin.
Kidney	*Uroporphyrin* (spectroscopically)	*Coproporphyrin* (spectroscopically)	—

CHART II.

Experimental findings in human case of Congenital Porphyrinuria (Petry), compiled from data of Fischer et. al. (1925).

Urine	Uroporphyrin I, 293°	Coproporphyrin I, 249–50°	—
Faeces	Nil.	Coproporphyrin I, 250°	—
Blood plasma	Nil.	*Coproporphyrin* (spectroscopically; no M.P.)	
Blood cells	—	—	Haematin.
Bones	Uroporphyrin I, 280–3°	Nil.	—
Bone (scapula)	Uroporphyrin I, 285° *Cu complex* (spectroscopically)	—	—
Bone marrow	*Uroporphyrin* (spectroscopically)	*Coproporphyrin* (spectroscopically)	—
Spleen	—	*Coproporphyrin* (spectroscopically)	—
Liver	*Uroporphyrin* (spectroscopically)	*Coproporphyrin* (spectroscopically)	*Protoporphyrin* (normal constituent, spectroscopically).
Bile	—	Coproporphyrin I 247°	—
Kidney	Uroporphyrin I 285°	*Coproporphyrin* (spectroscopically)	
Heart	—	*Coproporphyrin* (spectroscopically)	*Protoporphyrin* (spectroscopically).
Intestine	Nil.	*Coproporphyrin* and Cu complex (spectroscopically)	—
Pancreas	*proby. Uroporphyrin* (spectroscopically)	—	—
Muscle	—	*traces Coproporphyrin* (spectroscopically)	*traces Protoporphyrin* (spectroscopically).

DISCUSSION.

It will be seen from the accompanying charts that the investigation of this bovine case of congenital porphyrinuria has provided much additional information concerning the chemical nature and distribution of the pigments present in this disease. Thus, in the examination of the Petry material, the identity of the pigments (to which series they belonged, by melting point determinations) was achieved by Fischer and his collaborators (1925) in the case of the urine, faeces, bones, bile and kidney, these all proving to be series I pigments. As a result of the present investigation, however, the pigments have been identified by melting point as series I pigments in the following additional tissues; the blood cells and blood plasma, bone marrow, spleen, and liver. In general, the picture of the disease followed closely that seen in the man Petry.

The following four points, established among others, would seem to merit particular emphasis:—

(1) The bovine case of congenital porphyrinuria here studied exhibited clinical photosensitisation. It is thus brought into line with the chronic form of the disease in humans.

(2) Coproporphyrin I has been isolated from the blood plasma and erythrocytes, thus supplying a basis for explanation of the photosensitivity observed.

(3) The urine has been shown to contain uroporphyrin together with coproporphyrin I. No haemoglobin was present (compare Schenk, 1902).

(4) The bone marrow was rich in coproporphyrin I occurring with uroporphyrin. No protoporphyrin was detected.

Location of various Events in Pigment Metabolism and Modes of Excretion.

It is generally conceded that in the adult, haemoglobin is formed principally in the red bone marrow and that its transformation into bilirubin takes place in the cells of the reticulo-endothelial system, the liver playing an important part in this operation. As to the intermediate stages of both synthesis and degradation and the localities in which these changes take place, practically nothing is known. Thus, it is not proven that porphyrins form an obligate step in the normal synthesis of haemoglobin, although Borst and Königsdörfer (1929) are inclined to favour this view on account of their detection of protoporphyrin in the erythroblasts present in the red marrow, an observation in agreement with the findings of others. It has not been demonstrated, however, that the protoporphyrin here concerned belongs to the III series of isomers. However probable this might seem on general grounds, it can not be assumed as a basis for speculation, especially as a small quantity of protoporphyrin occurs normally in the erythrocytes and this would appear to belong, rather, to the I series.

The stages of the transformation of haemoglobin into bilirubin are just as uncertain. Iron must of course be eliminated at *some* stage but does this occur *before* or *after* the porphyrin ring is opened?

A reply to this question is possibly to be afforded by comparison with the system studied by Lemberg (1935) and would indicate an opening of the ring *prior* to the removal of iron, so that true porphyrins would at no stage be represented.

Warburg and Negelein (1930) had found that pyridine-haemochromogen in presence of hydrazine and molecular oxygen at 50° was converted into an iron-containing green pigment, exhibiting a characteristic absorption spectrum and which these authors regarded as a " green haemin ". Lemberg established the relationship of this substance to the bile pigments and was able to elucidate the course of the reaction. Pyridine haemochromogen (Fig. 22 No. I) is first converted by oxidative rupture of the ring system into a verdohaemochromogen (No. II) which still retains the central part of the molecule intact. The scission occurs at the same point as in the physiological formation of bile pigment from haemoglobin and these verdohaems are to be regarded as iron salts of isobiliverdins. The latter are unstable and if set free, rearrange by migration of a hydrogen atom from the pyrrole nitrogen of ring III to the >CO group of ring IV. The action of hydrochloric acid upon verdohaemochromogen thus consists in the removal of pyridine and oxidation to verdohaemin.

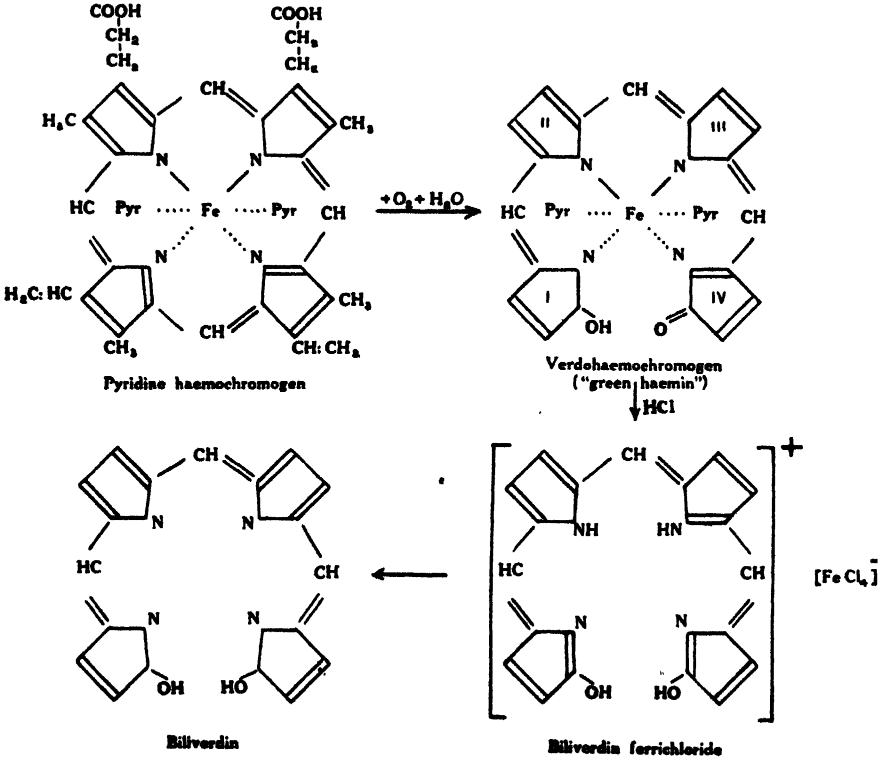


Fig. 22.—Scheme, after Lemberg, illustrating transformation of pyridine haemochromogen into biliverdin.

This is then split into ferric chloride and *iso*biliverdin which rearranges to give biliverdin. Esterification of the carboxyl groups takes place simultaneously and the $FeCl_4$ salt of the ester crystallises out (No. III).

It is quite conceivable that a similar course of events may be followed during the conversion of haemoglobin, or globinhaemochromogen, into biliverdin in the living cell. The difference between bilirubin and biliverdin is, of course, only the state of the methine carbon atom, uniting pyrrole rings II and III, the grouping being $-CH_2-$ in the case of bilirubin. Lemberg is of opinion that biliverdin is most probably the first of the two pigments to arise in vivo. (Lemberg and Wyndham, 1936).

Haemoglobin breakdown, would not, under these circumstances, require the formation of porphyrin at any stage and it is significant as will be pointed out below, that in none of the simple haemolytic anaemias is a significant increase in porphyrin excretion ever observed.

With regard to the site of the formation of the abnormal pigments found in congenital porphyrinuria, it would seem, from an inspection of the present data, that there is fairly strong evidence to show that coproporphyrin I, and probably uroporphyrin also, arises in the bone marrow. The spleen may present a second locality of coproporphyrin synthesis. Such a distribution would be consistent with the viewpoint that coproporphyrin I arises as a by-product or anomaly during the attempted synthesis of haematin.

The conversion of coproporphyrin into uroporphyrin is a simple chemical matter merely involving the addition of four carboxyl groups. Uroporphyrin may thus arise secondarily from coproporphyrin and would appear to do so most probably in the bone marrow and spleen and possibly also in the kidney. That this latter organ is chiefly responsible for the change would be a tempting hypothesis. Uroporphyrin, being highly hydroxylated, is eminently suitable for urinary excretion just as coproporphyrin is more suited for excretion in the bile, and it is somewhat difficult to understand how so great quantities of uroporphyrin can be found in the urine when the circulating blood contains coproporphyrin but only traces of uroporphyrin. Rabbits' urine normally contains some uroporphyrin (Fischer and Zerweck, 1924, a; Stockvis, 1873; 1895).

The deposition of uroporphyrin in the bones is explicable on account of the very great affinity which calcified structures show for this porphyrin. Thus Fränkel (1924), was able to show that small amounts of uroporphyrin injected into normal growing guinea-pigs coloured the bones in exactly the same way as is seen in congenital porphyrinuria, whilst other porphyrins were ineffective or only effective when administered in large amounts. It is understandable, therefore, that small quantities of uroporphyrin existing in the blood stream would rapidly be taken up and fixed by the bony skeleton.

The bile contained no uroporphyrin but was rich in coproporphyrin whilst in the liver this latter pigment could not be detected. Since the liver was functionally sound (no icterus or marked increase in urinary urobilin) it is permissible to conclude that it may excrete coproporphyrin from the blood stream with a high degree of efficiency into the bile. The urine also contained an amount of coproporphyrin I above the normal.

A transformation of coproporphyrin I into bile pigment would seem to be excluded on account of the isomerically different configurational relationship of the pigments in question and, moreover, the work of Lemberg discussed above renders improbable the intrusion of a porphyrin stage in the haemoglobin-biliverdin transformation. The bilirubin prepared by Fischer et. al. (1925) from Petry's bile was in no way abnormal.

TYPES OF ANAEMIA AND PORPHYRIN EXCRETION IN VARIOUS STATES.

Duesberg (1931) has critically compared the histological and chemical findings in different anaemic conditions, and as a result he is led to postulate the existence of at least two fundamentally distinct types of anaemia, that accompanied by increased regeneration processes and a type characterised by " impaired " or " disordered " regeneration.

Thus, whilst the appearance of the bone marrow was in all cases compatable with active erythropoetic efforts, there were in the former group of disorders signs of regenerative changes in the blood but no evidence of increased porphyrin excretion in spite of pronounced red cell destruction. Anaemias classed as belonging to the type with disordered regeneration were invariably accompanied by porphyrinuria. The following table will make this clear:—

Increased regeneration.	*Disordered regeneration.*
Increase in number of reticulocytes and increased oxygen consumption of blood. No porphyrin excretion.	Signs of regeneration in blood lacking. Porphyrin excretion.
Distilled water anaemia.	Lead poisoning.
Phenylhydrazine anaemia.	Sulphonal poisoning.
Haemolytic icterus.	Pernicious anaemia.
Saponin poisoning.	Congenital prophyrinuria.
Sodium nitrite poisoning.	
Anaemia by blood-letting.	

In the opinion of the writer, the differentiation should have been carried still further by a subdivision of the group in which porphyrin is excreted. Porphyrin elimination is only the objective end-result of a disturbance *at some point or other* of the normal pigment metabolism. The porphyrins are different in different types of intoxication. Firstly let it be again emphasised that simple haemolysis is unaccompanied by any significant degree of porphyrinuria even when the anaemia is intense as in phenylhydrazine poisoning, a fact which favours Lemberg's view of the direct formation of bile pigment from haematin, without passing over a porphyrin stage. All workers upon congenital porphyrinuria are agreed that the disturbance is not principally traceable to haemolysis.

Lead intoxication has, as a general rule, been regarded as leading to an anaemia of haemolytic origin, the excretion of a pigment derived from blood, latterly identified more exactly as coproporphyrin belonging to the III series, being cited as evidence for such a pathogenesis. To the writer this appears erroneous. Haemolysis does not lead to porphyrin excretion and it seems far more likely that in lead poisoning there is an interference with the processes of synthesis, presumably a failure to complete the stage of *incorporation of iron* into the porphyrin complex and thus the pigment becomes physiologically useless, even dangerous, and is excreted [compare Naegeli (1931), " Das Blei ist, trotz der basophilen Punktierung der Erythrocyten, kein Blutgift, es ist ein Knochenmarksgift, das die Erythropoese schädigt."]. There is no marked excretion of uroporphyrin in lead poisoning, it will be noticed; the quantity of protoporphyrin in the erythrocytes is, however, increased (Van den Bergh, Grotepass, Revers, 1932).

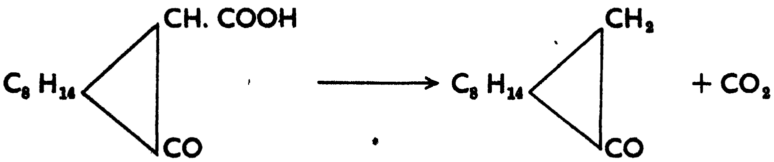
Sulphonal intoxication presents a different picture. The anaemia is never so marked as in the case of lead poisoning and may even be absent (in experimental cases). The bone marrow is very hyperaemic and shows signs of erythropoetic activity, the number of erythroblasts, however, being again smaller than in lead poisoning. The porphyrin excreted in sulphonal intoxication is chiefly uroporphyrin I together with some coproporphyrin, i.e. pigments belonging to the abnormal or unphysiological isomeric series, not series III pigments as in lead poisoning, and it is significant that the anaemia is frequently rapidly compensated. There would, in this instance, appear to be an interference with pigment synthesis of such a nature that useless by-products are produced and excreted, *without, however, the normal line of chemical elaboration being interfered with.* A similar state of affairs is pictured by the writer as holding in chronic congenital porphyrinuria. Pernicious anaemia (excretion of coproporphyrin I in faeces but disappearance after liver therapy, Watson, 1935, a) would seem to possess several features in common with the above condition.

A Provisional Theory of Pigment Metabolism.

Whilst our meagre knowledge concerning the intermediate stages in blood synthesis and breakdown renders difficult any comprehensive theory of pigment metabolism, it is nevertheless possible and extremely helpful to construct schemes which illustrate the broad features of these processes. Such a scheme has been put forward by Whipple (1922) but this, of course, was not particularly concerned with the formation and excretion of porphyrins in diseases such as those under discussion. As a result of the present work upon congenital porphyrinuria and taking into account other pertinent data, a scheme has been evolved which, it is felt, is capable of affording explanations of the main facts observed.

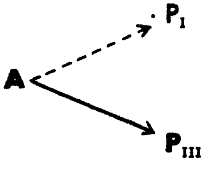
Thus, if it is assumed that coupling of substances containing pyrrolic groups and leading to the formation of porphyrins takes place in the erythropoetic tissues (bone marrow, etc.) as a normal step in the synthesis of haemoglobin, one can postulate that the possibility exists, on purely chemical grounds, of the elaboration in roughly equal quantities of four sets of isomers depending upon the relative positions of the substituent groups as in the four aetioporphyrins. Such would be the outcome of an in vitro chemical synthesis. However, since the available raw materials (probably derivatives of proline or oxyproline) are themselves asymmetric and only one type of isomer is presented for the synthesis, it is conceivable that the posibilities might be limited by one half, that is to say, isomers belonging to only two series could be produced. Of the four aetioporphyrin series, only derivatives belonging to the I and III series are found in nature, and the former only in relatively small quantities.

Now, biological syntheses are almost invariably catalysed by enzymes or enzymic systems, themselves asymmetric, and producing an overwhelming preponderance of a certain optical or configurational isomer, together, perhaps, with traces of its enantiomorph. For example, the naturally occurring amino-acids are, with the exception of glycine, all optically active molecules and the enzymes concerned in their intermediary metabolism have very little if any action upon the non-physiological isomers. Similarly, a striking degree of specificity obtains among the enzymes acting upon the purine bases, position isomers belonging to a simple series. A counterpart in pure chemistry to this specificity of enzyme action is to be found in the decomposition of d- and l-camphocarboxylic acids in aqueous solution as catalysed by nicotine. Normally, in the absence of any catalyst, the rates of break up of the two isomers are identical: —



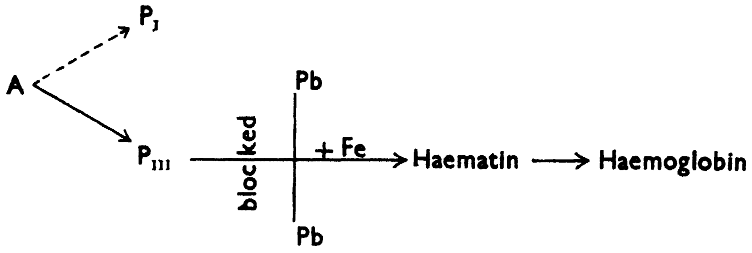
but when l-nicotine is added the relative rate of decomposition of the d-acid is markedly increased [Fajans (1910)].

One can therefore picture the events occurring in the bone marrow in the following way, where A represents the supply of asymmetric raw material and P_I and P_{III} the isometric porphyrin end-products produced. Since the change $A \longrightarrow P_{III}$ is selectively catalysed, it is shown in bold type whilst a dotted line indicates the production of small quantities of the by-product P_I

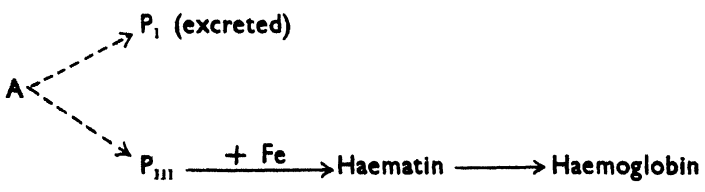


Protoporphyrin is probably the first formed and copro- and uroporphyrins arise from this as the result of further changes. The small quantities of series I porphyrins normally occurring in both animals and plants are thus to be regarded as quantitatively inferior and apparently useless by-products of the synthesis of the series III blood pigments.

Any interference with the elaboration of III series porphyrins into haematin would result in an anaemia accompanied by the excretion of coproporphyrin III but without any appreciable increase in the quantity of I series pigments normally present. This is precisely the state of affairs encountered in lead poisoning and it is understandable that compensation can only be effected, with the utmost difficulty, by an acceleration of the whole process of erythropoesis. Figuratively one may indicate the site of disturbance in lead poisoning thus:—



In congenital porphyrinuria (and possibly also pernicious anaemia), on the other hand, it would appear, as a reasonable explanation, that the disorder in pigment metabolism is due to a failure or inhibition of the selectively catalysed enzyme reaction $A \longrightarrow P_{III}$ thus resulting in the formation of P_I and P_{III} in quantitatively comparable amounts. As a result, to compensate for the threatened anaemia, and supply sufficient P_{III} for the requirements of haemoglobin formation, the whole level of porphyrin synthesis has to be raised and relatively large quantities of the useless series I pigments flood the organism and have to be eliminated. Congenital porphyrinuria may thus be analogous with alcaptonuria, cystinuria and other inherited errors of metabolism in that the organism is born lacking or deficient in a certain specific catalyst or enzyme necessary to complete a particular stage of intermediary metabolism. Sulphonal poisoning would appear to resemble congenital porphyrinuria in this respect that the selective action of the enzyme necessary for porphyrin III production is temporarily interfered with and an anaemia results, of no great intensity and easily compensated by an increased effort of activity on the part of the bone marrow but accompanied by a pronounced elimination of porphyrins belonging to series I.



The whole scheme of pigment metabolism in congenital porphyrinuria may thus be represented in the following way (see Fig. 23):—

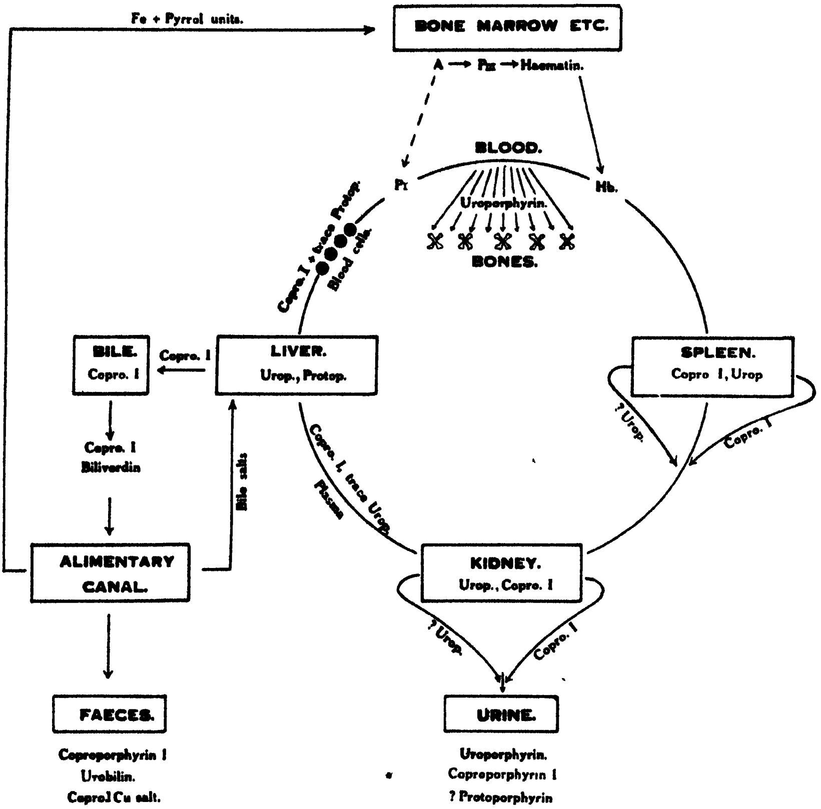


Fig. 23.—A provisional scheme of pigment metabolism with particular reference to the conditions obtaining in congenital porphyrinuria.

Incomplete and liable to error as the scheme may be, it should help, as a useful working hypothesis, to indicate further lines of investigation and by elaboration and improvement ultimately lead to a clearer understanding of the mechanisms underlying the synthesis of haemoglobin in the healthy subject and in various conditions of disease.

SUMMARY.

Of several living bovine cases of congenital porphyrinuria, discovered on a farm in Swaziland and all the progeny of a single pure-bred shorthorn bull (see Fourie, 1936), one animal was slaughtered for experimental purposes. This case, a castrated male, 2 years 4 months old, showed definite clinical symptoms of photosensitisation and passed a port wine-red coloured urine, exhibiting porphyrin absorption bands. The bones were found to be coloured a mahogany brown and on transverse section, concentric rings of lighter and deeper pigmentation were seen. The cartilages were normal.

Employing, in general, Fischer's methods, the individual organs and tissues were examined for porphyrins and pure crystalline materials (methyl esters) obtained as follows: Urine, *Uroporphyrin* (275-7°),*Coproporphyrin I* (233-5°); Faeces, *Coproporphyrin I* (243-4°) and its *Copper complex*; Blood plasma, *Coproporphyrin I* (243-4°); Erythrocytes, *Coproporphyrin I* (241°); Bones, *Uroporphyrin* (276-7°) and from a small sample derived from another case *Uroporphyrin* (273-4°); these esters had copper complexes 311-4° and 310-3° respectively. From the mother liquors of the main crystallisation was isolated a *Uroporphyrin* with ester M.P. 253-5° but yielding a normal copper salt. From Bone Marrow, *Uroporphyrin* (278°) and *Coproporphyrin I* (244-5°); Spleen, *Uroporphyrin* (278°); Liver, *Uroporphyrin* together with its *copper complex* (313°); Bile, *Coproporphyrin I* (237°). In other instances the yields of pure pigment were too small for identification by other than spectroscopic measurements (see chart in text of paper).

The significance of these pigments, belonging to the 1 series of porphyrins, is discussed in relation to normal haemoglobin synthesis and catabolism and the derangements of pigment metabolism occurring in disease and certain states of intoxication such as lead, sulphonal poisoning, etc. A suggestion is made as to the nature of the anomaly in congenital porphyrinuria and a provisional scheme of pigment metabolism mapped out.

I wish to thank Mr. G. Roets, B.Sc., for his generous assistance in the laborious task of working up the large quantities of material employed in this investigation and my colleague, Dr. Fourie, for the benefit of many discussions.

APPENDIX.

Examinations of Urine from Cases of Bilharziosis.

Since the disease bilharziosis is known to be accompanied frequently by the elimination of blood pigment in the urine and no reference could be found in the literature to any examination of such urines for porphyrin, it was deemed highly desirable to obtain firsthand evidence as to whether or not urinary porphyrin excretion is enhanced in this disease. The bovine cases of congenital porphyrinuria discovered in Swaziland were found on examination to be suffering also from bilharziosis. They have been treated for this condition and the infection apparently extinguished, nevertheless they continue to excrete large quantities of corporphyrin and uroporphyrin daily.

Through the kindness of Dr. F. G. Cawston of Durban, I was able to obtain about 250 c.c. of pigmented urine from an untreated human case of bilharziosis. A qualitative examination revealed the complete absence of uroporphyrin and the presence of traces of coproporphyrin in about the same quantity as is to be found in normal urine.

Some time later, Dr. A. Pijper of Pretoria was kind enough to procure for me 2·4 litres of urine pooled from three untreated human cases of bilharziosis then at the Pretoria General Hospital. This urine had all been passed during the period December 18-19th and was received and examined at the Laboratory on the latter date (Dec. 19th).

In colour, the specimen was brownish with a slight mahogany tint; bilharzia ovae were present. It was acidified with glacial acetic acid to a final concentration of 5 per cent. and then shaken twice with 1·5 litres of ether. Some difficulty was experienced on account of emulsification. The ether extract was washed repeatedly with water and the porphyrin transferred to 5 per cent. hydrochloric acid affording a reddish solution exhibiting the acid porphyrin bands. After filtration through cotton wool, potassium acetate was added and the pigment again extracted with ether. This solution exhibited the following absorption bands: 623·4; (568·1); 529·1; 497·6.

The porphyrin was transferred again to 5 per cent. hydrochloric acid and the solution shaken with chloroform which removed some pigment including a trace of porphyrin, almost certainly protoporphyrin. The residual acid had a pale pink colour and showed the following bands, agreeing with those of coproporphyrin: 592·0; (572·2); 549·1.

It was compared with a standard porphyrin solution made by dissolving 1 mgm. of pure coproporphyrin I tetramethyl ester in 2 c.c. of concentrated hydrochloric acid and, when saponification was complete, diluting until the volume was 100 c.c. and the final acid concentration 5 per cent. 1 c.c. of the stock solution had to be diluted to exactly 1·5 c.c. for the intensity of the absorption bands to match that of the urinary porphyrin solution, the total volume of which was 20 c.c. Colour intensity comparison in a calorimeter afforded a similar result.

∴ 20 c.c. of urinary extract contained $20 \times \frac{1}{150}$ mgm.

$= 0{\cdot}133$ mgm. porphyrin.

This quantity of pigment was derived from 2,400 c.c. of urine.

∴ 100 c.c. urine contain $\frac{0{\cdot}133}{24}$ mgm.

$= 5{\cdot}56\ \gamma$ porphyrin.

($1\ \gamma = 1/1000$ mgm.)

Schreus and Carrié consider an excretion of 0 to 60 γ of coproporphyrin per day to represent the normal range, although figures as high as 80 γ were also obtained in some insetances. Günther considers 400 γ per litre to be pathognomic. Assuming an excretion of 1 litre per day by the patients investigated (it would certainly not be higher in this hot climate), it will be seen that the content of coproporphyrin found falls within the normal range.

Of the acid urine left after ether extraction, 750 c.c. was filtered through a column of active alumina and the chromatogram worked up by a method shortly to be described. Only a trace of pigment was obtained showing an absorption spectrum. It could not be shaken from ether solution to 5 per cent. hydrochloric acid and most probably represented a metal complex of coproporphyrin, since the following absorption bands were seen in pyridine solution. 564·0; 533.

For the sake of comparison with the above results, 320 c.c. of urine from a porphyrinuric bovine (No. 7018) was worked up quantitatively for coproporphyrin. The result was as follows:—

Volume of final solution in 5 per cent. hydrochloric acid = 500 c.c.

1 c.c. of this diluted to 1·1 c.c. matched 1 c.c. of standard porphyrin solution.

∴ total quantity of coproporphyrin present = 8·5 mgm.

or 1·72 mgm. per 100 c.c. of urine.

In concentration alone, this is 300 times the quantity found in the human bilharzia urine or in terms of daily excretion very considerably more (say approximately 1 to 2 thousand times as much). In addition, uroporphyrin was also present, of course, in considerable quantity in the bovine urine.

I wish to express my sincere thanks to both Dr. Cawston and Dr. Pijper for their kindness in placing the specimens at my disposal for examination.

NATURE OF THE UROPORPHYRIN IN THE BONES FROM THE BOVINE CASES OF CONGENITAL PORPHYRINURIA.

As recorded in the accompanying article on page , the melting points of the uroporphyrin ester isolated from the bones and urines of those cases examined are lower than that recorded by Fischer for pure Uroporphyrin I methyl ester. In addition, a fraction was isolated from the mother liquors of the main crystallisation of the bone ester which appeared to be homogeneous and to have a greater solubility in methyl alcohol than the main product and also a melting point as low as 253-5°. The copper complex, prepared in the usual way, had melting point 300°.

Some months after these observations were made, the writer visited Europe and there learnt that Waldenström (1935, Deut. Archiv. Klin. Med. Vol. 178, pp. 38-49) and also Mertens (1936, Zeit. physiol. Chem. Vol. 238, p. I) had independently and almost simultaneously succeeded in isolating Uroporphyrin III from urines in cases of *acute* porphyrinuria, the structure being proved by decarboxylation to coproporphyrin III. The melting point of Uroporphyrin III octamethyl ester was given as 255° to 258°; copper complex 304°. Uroporphyrin I was not present in these cases. I had with me a specimen of Uroporphyrin (M.P. 268°), very small in quantity (4·6 mgm.) but Dr. E. Mertens of the Eppendorfer Krankenhaus, Hamburg, very kindly offered to carry out the decarboxylation of this sample according to the technique previously used and to compare the copper complex of the coproporphyrin ester with that previously obtained by her from Uroporphyrin III. The experiment yielded only the derivative of the series I ester and by the removal of the copper and crystallisation, the identity of the porphyrin (ester M.P. 245°) with coproporphyrin I was further confirmed. Such a result indicated that the original uroporphyrin sample of low melting point was an impure uroporphyrin I or possibly uroporphyrin I in loose combination with some accompanying material. Compare

Fischer and Duesberg (1932) whose preparation of M.P. 269° also only yielded coproporphyrin I on debarboxylation. Shortly afterwards, Fischer and Libowitzky (1936; Zeit, physiiol. Chem. Vol. 241, pp. 220-2, Nachschrift bei der Korrektur) claimed to have separated a low melting point uroporphyrin ester derived from Petry (? bone), M.P. 286°, by means of adsorption analysis into a uro-III ester of M.P. 261° and a uro-I ester of M.P. 302° (uncorr., 311° corr.) The technique is not described, neither was the identity of the uroporphyrin III ester proved by degradation to the corresponding coproporphyrin.

The results of Fischer and his co-workers would thus seem to conflict with the present writer in whose low melting point preparation, as stated, Dr. Mertens found only uroporphyrin I unless uroporphyrin III was also present and coproporphyrin III being so much more difficultly crystallisable than the I isomer had not been detected in the products of decarboxylation. It must be admitted, however, that the specimens in the present instance had not been purified by chromatographic adsorption.

I wish to express my sincere thanks to Dr. E Mertens for her very friendly and valuable collaboration and foi permission to make use of the results she obtained in this way. My thanks are also due to Professor O. Schumm, the Principal of the Institute where the experiments were actually carried out.

CHROMATOGRAPHIC SEPARATION OF THE UROPORPHYRIN INTO TWO ISOMERS.

Attempts to separate the unesterified porphyrin chromatographically having given somewhat disappointing results, (these experiments will be recorded later) attention was directed to the treatment of the esters by the chromatographic method. In the search for a suitable solvent, the observation was made that uroporphyrin octamethyl ester is easily soluble in hot dioxan (diethylene dioxide) but separates from this solvent on cooling in fine crystalline form. It is also possible to effect a high degree of purification by two or three repeated recrystallisations from this solvent since the low melting isomer is apparently much more soluble and remains largely in the mother liquors. Thus, a specimen of uroporphyrin ester from bovine urine which had from chloroform-methyl alcohol a melting point practically constant at 278° was found to melt after one crystallisation from dioxan at 289-290° and after a second recrystallisation at 291-293°.

Dioxan is a solvent eminently suited to the chromatographic method; alumina (Merck's ' nach Brockmann ') was found to be a better adsorbent than either calcium carbonate or talc. The above specimen when put through the column diffused fairly rapidly and homogeneously downwards being washed out by fresh dioxan and leaving a small mahogany coloured ring near the top of the column. The mother liquors of the first recrystallisation, similarly treated, gave a homogeneous rapidly diffusible fraction, crystallised by addition of hot methyl alcohol to the dioxan solution, M.P. 292-293°, and a narrow ring of pigment which was eluted by pyridine or by chloroform containing acetic acid (methyl alcohol containing 5 per cent. by volume of sulphuric acid is a still better eluting agent) and crystallised in the usual way. It separated in the typical uroporphyrin form, had M.P. 260° and gave the following absorption bands in chloroform:

625·8; 572·1 *maximum;* 535·0; 500·0.

This material would appear to be the ester of Uroporphyrin III. When similarly treated, the uroporphyrin, separated from the bones of the bovine case reported upon in this paper, (M.P. 276-7°) was resolved into pure uroporphyrin I the melting point of which could not be raised above 293° by repeated adsortion, and a small quantity of the other isomer M.P. 261° which it would appear is uroporphyrin III. The discrepancy between the various melting points recorded in the literature would thus be explicable upon the basis that small amounts of uroporphyrin III accompany the I series porphyrin in the congenital form of the disease.

The coproporphyrin ester which had been obtained from the porphyrinuric bile (M.P. 237°) was dissolved by warming in a little dioxan and filtered through an alumina column. The chromatogram on development with dioxan afforded a sharply defined, narrow ring near the top and a more rapidly diffusing fraction. This latter was coproporphyrin I (M.P. 246-8°) whilst the small amount of pigment retained more tenaciously appeared also to be a coproporhyrin.

Spectrum in chloroform: 626·2; 563·5 *maxmum;* 529·5; 496·7.

Further work in this direction is in progress.

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Section VI.

Animal Husbandry.

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A Contribution to the Study of African Native Cattle.

By H. H. CURSON, Section of Anatomy and R. W. THORNTON,
Director of Agriculture, Basutoland.

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APPENDIX.

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(1) A questionaire was *not* sent to the countries indicated by an asterisk.

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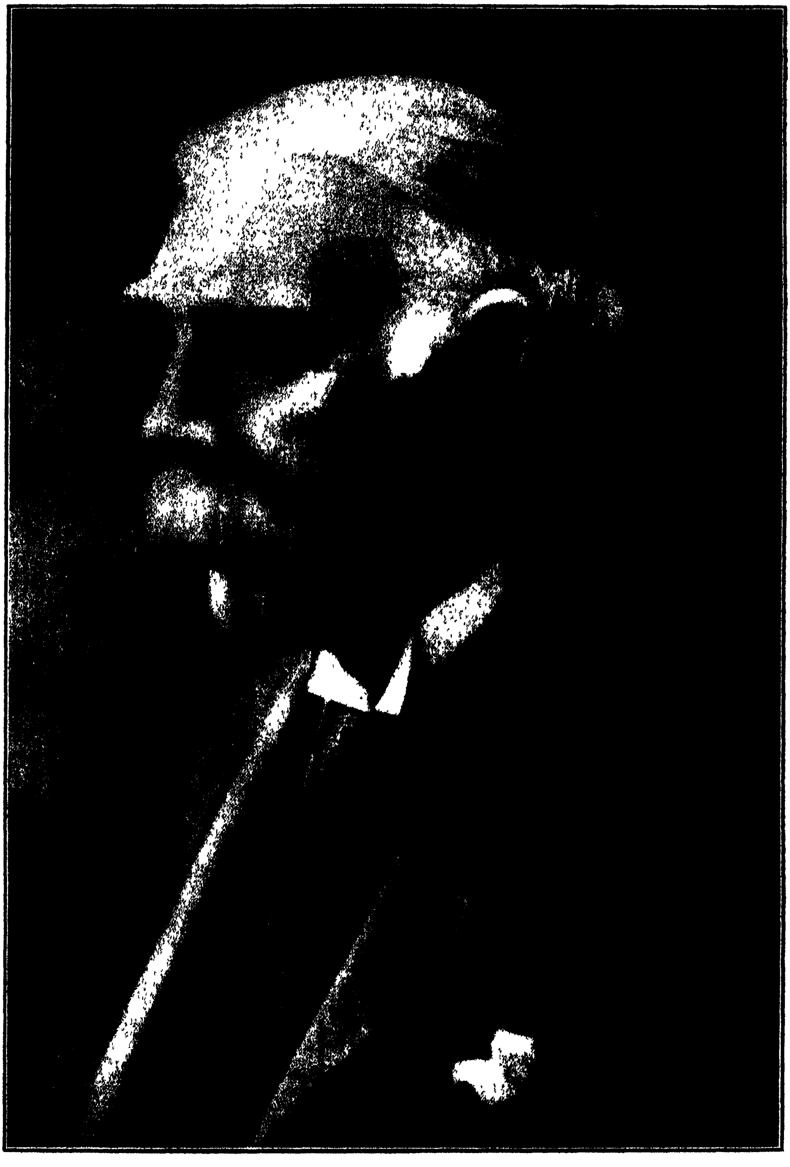

A. C. MACDONALD (1865-1926) who first recognised the Afrikander as a native beast. Archibald Campbell Macdonald was in the service of the Department of Agriculture, Cape Colony, from 1889-1902. He then became Deputy-Director of Agriculture, Transvaal, from 1903 to 1907. From 1st July 1907, to 11th March, 1920, he was Director of Agriculture, Kenya. He died at Kabete on 6th July, 1926 (H. H. Brassey-Edwards, M.R.C.V.S.)

PREFACE.

ONE of the most striking features concerning Cattle Husbandry in Southern Africa is the fact that little or nothing has been done by Europeans to improve indigenous stock by selection, especially in view of the environmental difficulties experienced in farming, *e.g.* drought and disease. This is all the more astonishing when one considers that in Great Britain and Holland, the original home of most white South Africans, skilful selection has led to the creation of some of the finest breeds in Europe. The practice in Southern Africa has been in the vast majority of cases to improve native stock by grading with European breeds, a measure which in the opinion of Bisschop (1934) leads to degeneration in the arid and semi-arid regions of the country. As it is, the greater part of the Union is no longer occupied by pure indigenous cattle, but by nondescript herds destined, as in Egypt, to be absorbed ultimately into the Brachyceros type.

Four excellent examples are provided by South Africa alone illustrating the advantages of improvement by selection, namely, Afrikander cattle, Blackhead Persian sheep, Angora goats, and the ostrich.

The authors, both of whom have travelled fairly extensively in Africa and have for many years realised the potentialities of indigenous cattle, endeavour in this contribution to give an outline of the cattle types of Africa with their approximate distribution. This after all is the basis of cattle husbandry whether improvement is to be effected by selection or by grading up.

A classification based on conformation now being available, a new approach suggests itself, namely the distribution of blood groups and its relationship with the physical classification. This and other fundamental problems, particularly in regard to genetics and nutrition, offer much scope to those who have Native Cattle Husbandry at heart.

In conclusion, we wish to express our indebtedness to the officials of the various Administrations for their co-operation in the compilation of data. By their precise replies to the Questionaire, they have made the compilation of this article possible. Cordial thanks are due to the Secretary for External Affairs, Pretoria, for arranging the issue of the Questionaire and for translations, and to the Secretaries of Agriculture and Native Affairs, Pretoria, for their support. Particular appreciation is expressed to the Director of Veterinary Services (Dr. P. J. du Toit) for allowing his staff to participate in this compilation, chiefly in connection with the typing and photography. The assistance of others who rendered help *e.g.* by the loan of photographs is acknowledged in the article, but a special word of thanks is due to Dr. A. D. Thomas for his aid in French translation.

R. W. T.
H. H. C.

Onderstepoort.

CHAPTER I.

PRELIMINARY.

INTRODUCTION.

The object of this paper is to make available the data collected from (*a*) certain African territories and (*b*) certain extra-African countries as the result of a Questionaire issued by the Secretary of Native Affairs (his file N.A. 13/327) through the Secretary of External Affairs in 1931.(⁴) The replies received from the African countries form the basis of Chapter II and those from outside Africa have been dealt with under Chapter III. Additional data, however, have been incorporated.

The Questionaire was drawn up by one author (R. W. T.) and replies have been assembled into narrative form by the other author (H. H. C.) who received the files in November, 1935.

Although at present receiving but scant attention, the problem of Native Cattle Husbandry really interests three State Departments, *viz.*, the Division of Veterinary Services which is concerned chiefly in conformation and relationship to disease, the Division of Animal Husbandry in the economic position from the European standpoint and the Department of Native Affairs which considers the matter naturally more from the native aspect.

A definite policy has not yet been laid down, but it is hoped that one of the results of this contribution will be to focus attention on the subject and thus hasten the formation of some policy. This obviously will be to the benefit of South Africa as a whole.

HISTORICAL OUTLINE.

As has been emphasised frequently, but little attention has been paid in South Africa to Native cattle as compared, for example, with the position in India.(⁵)

After the Anglo-Boer War of 1899-1902, A. C. McDonald of the Transvaal Department of Agriculture (see Frontispiece) showed some interest in Afrikander cattle. Not only did he select, with the assistance of the Repatriation Department, a herd of 50 head as a nucleus for further improvement(⁶), but he expressed himself as being " rather inclined to the idea that the Afrikander cattle are

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(⁴) At a meeting held in the office of the Director of Native Agriculture, Pretoria, in 1930 and at which both authors were present, it was agreed that a preliminary step in any investigation in native cattle should be the issue of a Questionaire. Others present were Professor A. M. Bosman (University of Pretoria) and Mr. G. P. Lestrade, Ethnologist, Native Affairs Department, Pretoria.

(⁵) Even in Africa [Pierre, of French West Africa (1906), and Carlier, of Belgian Congo (1912)] detailed attention was given to this subject over 20 years ago.

(⁶) The result of this experiment is not known. It is believed the cattle went to Potchefstroom School of Agriculture.

descended from one or other of the breeds which were brought down from North Africa by the Native tribes ". (*Transvaal Agricultural Journal*, October, 1904.) This view is now generally accepted. At the same time Sir Arnold (then Dr.) Theiler collected the skulls of indigenous cattle and sent them to Professor Conrad Keller of Zurich for craniological study. The result of this investigation appeared as a thesis in 1911, Hendrik Molhuysen of The Hague having worked up the material for a doctorate at the University of Zurich (Molhuysen, 1911). Subsequently Sir Arnold Theiler with Daniel Kehoe, M.R.C.V.S. (1888-1928) made observations on native cattle, but unfortunately the notes were not published and are now not available. Again in 1922 Sir Arnold Theiler made an effort to develop this important branch of Animal Husbandry. He endeavoured to arrange that an official at the Potchefstroom School of Agriculture should " undertake anatomical research into the various breeds of cattle . . . . under " his control; but owing to departmental difficulties the scheme lapsed. In the meantime, however, a few skulls had been received at Onderstepoort from South-West Africa (Groenewald and Curson 1936) and Tanganyika (Curson 1936) and these now form part of the Osteological Collection.

In 1927 one of us (H. H. C.) was appointed Professor of Anatomy in the University of South Africa (Transvaal University College) and the Director of Veterinary Services kindly allowed the investigations to proceed. In 1929 the second author was appointed Director of Native Agriculture of the Union of South Africa and a commencement was made in 1930 in the establishment of a herd of white cattle (*Nyonaipumuli*) at Nongoma, Zululand. The greatest impetus, however, was given to the investigations in 1933 when Dr. H. Epstein of Welverdiend near Potchefstroom made available his notes on researches undertaken in Europe in regard to the racial history of African cattle(⁷).

From time to time articles and references to native cattle in the Subcontinent have appeared in the literature (Nobbs of S. Rhodesia, 1927, and Duerst, 1931) (⁸). These have in the main been referred to in the papers published since 1930 from Onderstepoort, including a list of references on the Afrikander (Curson and Bisschop 1935).

The Migration Routes of Cattle in Africa.

It was Epstein who in 1933 first linked the scattered facts and put forward a working hypothesis in regard to the origin of our cattle, associated with, of course, the various human migrations that have taken place since the earliest times.(⁹)

(⁷) Unfortunately Dr. Epstein's monograph on *The Origin of Africa's Indigenous Domestic Animals* has not yet appeared in print.

(⁸) Duerst should certainly be consulted.

(⁹) While unfortunately Epstein's researches have not been published *in toto*, an idea of his views may be gained from a study of Epstein (1933), Curson and Epstein (1934), and Epstein (1934). He no longer holds the view (letter of 10.4.36 to Director of Veterinary Services) that " Hamitic Longhorn cattle are pure descendants of *Bos primigenius* Hahni, but a mixture.

In short, it is believed that the first cattle to be domesticated in Africa were " the giant horned wild oxen of the Nile Valley called by Hilzheimer *Bos primigenius* Hahni, *nova sub-species Hilzheimer* ".

Then at the end of the Neolithic era, there entered Lower Egypt from Asia . . . cattle of an entirely different type, namely, the Brachyceros or Shorthorn. These accompanied the first invaders of Mediterranean stock(10), who on their arrival compelled the original tribes (of Negroid stock) to retreat westwards. The migration of the people of the Mediterranean stock no doubt occupied many centuries, and it was notable for the arrival, about the end of the 3rd pre-Christian millenium, of Semitic tribes, who were accompanied by lateral horned cattle of zebu type (the present day Afrikander). This invasion further disturbed the Negroid people in what is to-day Upper Egypt and Abyssinia; and many tribes were compelled to seek safety by retreating southwards *viz.*: the Bushmen, Hottentots and Bantu.

In consequence of the first invasion some of the original Hamitic Longhorn cattle were taken westwards along the Mediterranean littoral to what is now Morocco. There the stream of migration divided, one branch proceeding north into Spain and Portugal and beyond, and the other south into what are to-day French West Africa, and the various territories along the Gulf of Guinea, but particularly Liberia and Nigeria. Descendants of cattle which were taken into Europe are seen to-day in the Raza de Barroza, Raza Minhota, and Raza Alentejana of Portugal and the Andalusian cattle of Spain. Da Costa (1931), however, considers the Raza Alentejana a relative of the Afrikander. From the Iberian Peninsula Brazil imported cattle and the Franqueiro breed there represents the Hamitic Longhorn. Great Britain too, has representatives in the Black Cattle of Wales, West Highland Cattle and Herefords. While the Hamitic Longhorn has disappeared from North-East Africa, Sir Harry Johnston (1906) encountered descendants in Liberia and judging from the cattle of French West Africa and Nigeria, there is but little doubt that the present West African Zebu, with its large lyre horns and characteristic head, contains a large proportion of Hamitic blood.

As mentioned above, the first invaders of Egypt brought cattle of shorthorn type, which in time displaced the Hamitic Longhorn in Lower Egypt and even during the period of the New Kingdom (1580-945 B.C.*) they were dominant. In the course of time their owners were also compelled to seek pastures new, and again the path to the west along the Mediterranean was selected. As before, when opposite Gibraltar, the stream divided, one to the north into Spain,

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(10) At the time of the Pharaohs, except for ~~the~~ " Egyptian aristocrats of the Pyramid Age " (who were of Alpine stock), the inhabitants of Africa belonged to Elliot Smith's Negroid type [Perry (1935)]. Elliot Smith's types are Nordic, Mediterranean, Alpine, Mongolian, Negro, and Australoid [*Human History* (1933)].

* Yahuda (1934), p. xvii.

France and Great Britain and the other south along the shores of the Atlantic until the southern arm of the Gulf of Guinea was reached after centuries of travel. Representatives of this type are to be found to-day not only along the Gulf of Guinea as far south as the Cameroons, but around the northern part of Afrcia as far east as Eritrea.

In the meantime had arrived, as mentioned above, the Lateral-horned Zebu. These cattle had a great influence on the Hamitic Longhorn occurring in Upper Egypt and Abyssinia, in fact as a consequence of the intermixture between the two stocks was created the Sanga type, the most common to-day in Africa south of the Sahara. Fortunately as a result of representatives of the Lateral-horned Zebu stock coming into possession of the people now called Hottentots, cattle of this type were saved for posterity. The Hottentots being in the van of the human stream which migrated southwards, kept their herds pure. It can be imagined that the Hottentots would follow the Great Lakes and when south of Nyassa would bear to the west and so reach what is to-day the South-West Africa Protectorate. Sir Harry Johnston believes that " the first Bantu invasion from across the Zambesi " (quoted by Prof. L. F. Maingard *S. African Jl. Sc.* 1929, p. 845) took place about 700 A.D., sc that the Hottentots must have reached Southern Rhodesia before this date. When the Hottentots arrived on the shores of the Atlantic they worked their way south around the Cape of Good Hope and on the arrival of the Dutch colonists in 1652, the advance guard with its numerous herds had already entered what is now the Eastern Province.

Above have been described the probable migration routes of the three parent stocks of African cattle, *viz.*: the Hamitic Longhorn, Brachyceros ,and Lateral-horned Zebu. Reference will now be made to the likely paths taken by the owners of the Sanga cattle, established in Abyssinia and Upper Egypt by the intermixture of Hamitic Longhorn and Lateral-horned Zebu types.

Following comparatively close on the heels of the Hottentots were tribes which included the Bantu of to-day. This migration, accompanied by their herds of Sanga cattle either divided into western and southern streams soon after their departure from North-East Africa (probably Abyssinia), or left at different periods. In any case the western nomads passed through the southern part of the Anglo-Egyptian Sudan, and skirted the Glossina region of French Equatorial Africa until Lake Chad was reached. Here to-day are to be found representatives of Sanga cattle. The southern stream probably passed through Uganda as had the Hottentots, and followed the Great Lakes until the Zambesi was reached. There it can be imagined, the various tribes dispersed, some going west into Bechuanaland and Ovamboland, and others south into Southern Rhodesia and the Transvaal, and the remainder east into Portuguese East Africa and Zululand. As is well known, the advancing Bantu came into contact not only with the Hottentots, but also the European settlers in the Eastern Province of the Cape of Good Hope.

Epstein believes that the next derived type, the Shorthorned Zebu, reached Africa from Asia when the power of Persia was at its zenith and afterwards during the Arab invasion. He further considers that it represents the influence *in Asia* of Brachyceros on the Lateral-horned Zebu. Map I represents the Shorthorned Zebu as arriving only by sea, but this is not the case. While osteological evidence may support this view, a study of the hump, which is thoracic and musculo-fatty (in contradistinction to that of its supposed progenitor, the Lateral-horned Zebu) certainly does not do so. In any case the Shorthorned Zebu is a comparatively recent arrival and distribution along the East African coast as far south as the Zambesi was due primarily to Arabs and Indians engaged in commerce. At some period centuries ago a migration must have occurred in the direction of Nigeria from north of Kenya, for not only are there cattle of the Shorthorned Zebu type in the territories bordering on the Gulf of Guinea, but another West African Zebu, the Lyre-horned Zebu, represents an intermixture between the Hamitic Longhorn and Shorthorned Zebu types. Hitherto only five racial groups have been defined in Africa, but it is considered that in view of numbers and uniformity of type, the West African Lyre-horned Zebu deserves to be classed as a sixth group or type(11).

Before closing the matter of migrations it should be stressed that migration is still occurring,(12) the Shorthorned Zebu being the principal type concerned. A study of Map II of the distribution of native cattle shows that the Zebu of East Africa is gradually extending westwards from Northern Rhodesia through the Belgian Congo to the Sudan. In the reply to the Questionaire—see next section) received from the Cameroons it is learned that zebu cattle are of recent introduction into the north, having come through Northern Nigeria along with their owners from French West Africa.

In describing the cattle of the various African territories, the countries associated with the various migrations will be dealt with as follows:—

First and Second Migration Routes......	**Egypt, French North Africa, Gold Coast, Nigeria, and French Equatorial Africa.**
Third and Fourth Migration Routes......	**Abyssinia, Anglo Egyptian Sudan, Uganda, Kenya, Belgian Congo, Tanganyika, Northern Rhodesia, Southern Rhodesia, Portugese East Africa, South West Africa, and Union of South Africa.**
Fifth and Sixth Migrations.............	**East Africa to West Africa.**

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(11) A distinct polled type such as is featured by Keller in Ancient Egypt [Kronacher (1921)] need not be discussed here.

(12) The European partition of Africa has been the most effective check to tribal migration.

CLASSIFICATION OF NATIVE CATTLE.(13)

The indigenous cattle of Africa may be provisionally classified thus:—

Non-Humped.	*Humped or Zebu.*	
Hamitic Longhorn	1. *True Zebu* (Asiatic in origin)	1. Original *lateral horned Zebu*, now called Afrikander.
Brachyceros.		2. *Shorthorned Zebu*. Essentially of Asia, but well represented in East Africa.
	2. *Pseudo-Zebu* (African in origin)	3. *Sanga*, best represented in South Africa.
		4. *Lyre-horned Zebu*, best represented in West Africa.

In the main sub-division, the presence or absence of the hump, irrespective of situation and structure, is the determining feature. In the further sub-division into True- and Pesudo-Zebu, not only the geographical origin, but also the type of skull and presence or absence of bifid superior spines of the thoracic vertebrae (from 6th vertebra backwards) have been considered.

The True Zebu has most frequently a long coffin shaped skull and the orbital arches are not prominent. In addition the profile is generally convex, and the thoracic superior spines are bifid.

The Pseudo-Zebu is not only not so uniform in conformation, but the skull shows marked evidence of Hamitic Longhorn influence, being wide in the forehead and the orbital arches prominent. The profile is generally flat. In the Sanga the superior spines of the dorsal vertebrae vary greatly, being either single or bifid; but nothing is known regarding the Lyre-horned Zebu, since no osteological material has been examined. The status of the latter has been determined entirely by photograph, which although as valuable in some respects as a written description, is not sufficient.

Suppose the *type of hump* were used as the criterion for the sub-division of the Zebu, we should have:—

Cervico-thoracic and muscular hump....	1. Original lateral-horned Zebu. 2. Sanga.
Thoracic and musculo-fatty hump......	3. Shorthorned Zebu. 4. Lyre horned Zebu.

Of the two schemes the former appears to be the more suitable.

A Plea for Co-operation.—The only method of deciding which types occur in a territory is to inspect the cattle *in loco*. Failing this, the alternative method is to (*a*) procure photographs (lateral view) of typical bulls and cows, with particulars regarding the withers height and nature of hump *in each* case, and (*b*) obtain, if possible (i) the skull of a good representative of the type, both bull and cow, and (ii) a transverse slice of the hump, about 2 inches in width and taken vertically from the summit of the hump. The section of the hump, preserved of course in formalin, will at once indicate whether it was taken from *either* a Lateral-horned Zebu (Afrikander) or Sanga *or* from a Shorthorned Zebu or Lyre-horned Zebu. The hump of the Sanga resembles the Afrikander while that of the Lyre-shaped Zebu appears to differ in no way from its Shorthorned relative. No data are available concerning the hump in the crosses between the groups mentioned.

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(13) This classification does not entirely agree with that of Da Costa (1931).

The differences between the various types and sub-types are exceedingly well-marked, but in time when skull material is available it will be advisable to express certain features in a statistical manner. Not only will it be necessary to show the most constant features in measurements within the same group, the range of variation in a particular type or sub-type, but also the differences between the several classes.

A word is also necessary in regard to the measurement of cattle. The age, weight and sex must always be given and the following measurements, preferably in centimetres (*cm.*), are suggested:—

(*a*) Length of body, *i.e.* from point of shoulder to *Tuber ischii.*
(*b*) Height at withers. In the case of cattle with a hump the details including and excluding the hump should be given.
(*c*) Height at hookbones, *i.e.* in a line joining the *T. coxae.*
(*d*) Depth of chest, *i.e.* immediately behind elbow.
(*e*) Width of chest across back, and behind shoulder.
(*f*) Width of hookbones.
(*g*) Width between thirls, *i.e.* Femur, *Trochanter major.*
(*h*) Minimum width between pinbones, *i.e. Tuber ischii.*
(*i*) Heart girth.
(*j*) Length of head from summit of *Torus frontalis* to cranial end of nasal bone.
(*k*) Width between eyes at medial canthus.
(*l*) Length of croup, *i.e.* from *Tuber coxae* to *Tuber ischii.*

In points which do not lie in a line parallel to the vertebral axis, the measurement is not taken in a direct line but in projection.

QUESTIONAIRE.

While the Questionaire might have been inserted after the section Historical Outline, it is felt since much has already been written independently on migration and classification that it would be better if placed here.

" *Countries and Territories from which information is desired.*

India.
Egypt.
Morocco.
Sweden.
Brazil.
Northern and Southern Nigeria.
British and French Cameroons.
Belgian Congo.
Algeria.
South West Africa.
Northern and Southern Rhodesia.
Uganda.
Kenya.
Norway.
Tanganyika.
Argentine.

Introduction.

With a view to building up certain types of native cattle in native areas, the Department of Native Affairs of the Union of South Africa is desirous of securing the most complete information possible on all types of native cattle found throughout the continent of Africa, and also of such other types in other parts of the world which resemble in colour marking, conformation, or other special features, certain special types found in the Union. It is hoped by tracing the breed-history of certain types to establish to some extent the breeding lines to be followed with native cattle in South Africa, particularly with reference to the type of local or imported sire to be used.

The countries co-operating in furnishing the information requested will, if they so desire, be supplied with a complete copy of all the information secured with an analysis of the position in relation to South African cattle prepared by the officer responsible for the work in the Union.

General Questions.

1. If possible, give the origin of recognised types or breeds of cattle.

2. Are the breeds or types mentioned under (1) believed to be indigenous or were they imported? If the latter, when and from where?

3. State whether the breeds or types mentioned are humped or straight-backed.

4. Give a full description of the colour marking of each breed or type mentioned.

5. State whether polled, horned, or a mixture of both, with a description of the horns.

6. State whether the animals are of good beef type or milking type; or neither; or both.

7. State which of any breed described is supposed to be the original breed and whether such breed or type was founded on other types now extinct; and, if the latter, please supply a description, if possible, of such original extinct breeds or types as can be secured from paintings, rock drawings, and sculpture, etc.

8. State which breed or type was first domesticated, the approximate date of domestication, and whether still in use.

9. Please supply photographs in triplicate of typical specimens of both males and females of each breed or type dealt with.

10. Do any of the breeds or types described possess any special features such as slow maturity (say, five to seven years) or display extreme hardihood against adverse climatic conditions (such as great heat, drought), disease resistance, ability to range over great distances, etc.

11. Special mention must please be made and the fullest possible particulars given of any white cattle with dark points which exist in any of the countries mentioned.([14]) By dark points is meant black ears, muzzles, eye rings or eye lashes, black teats, black hoofs, or spotted or black markings below the knee and hock; also whether any animals answering this description have black skins covered with white hair and whether black spots show through the white hair causing a silvering or spotted appearance, particularly as the animal advances in years.

12. Which breed or type described is considered to be the most valuable and why.

([14]) This point is stressed, for one of us (R.W.T.) considers the colour of the coat, particularly white, an important point in the relationship of native cattle types.

13. Please give the territorial distribution of any type or types described in such manner as to permit of tracing the areas mentioned on a map. Please also give a description of the areas such as elevation and whether open highland, grass, bush, or forest country; or whether low-lying, hot sandy, marshy bush or forest country with heavy or low rainfall in each case.

14. Whether partially resistant to or immune from any disease such as nagana or other widely distributed and known trouble.

15. Any other information.

In addition to the information asked for under the foregoing general points, information on specific questions would be much esteemed from the following countries:—

India.

1. Is the Krishna Valley Indian Zebu any better as a beef or milk producer or in any way different in colour or conformation from the general type ot Indian Zebu?

2. Where can the best strain of beef and milking zebu be obtained in India and at what price for males and females?

3. Can any information be supplied as to when cattle were first domesticated in India and whether the present-day Zebu is considered to be descended (without the infusions of other blood) from the original stock.

Egypt.

1. When were cattle first domesticated in Egypt? Did domestication take place during the third Egyptian Dynasty, say, 5,000 to 4,800 B.C. or at an earlier date?

2. Do desert or other rock drawings, sculptures, or other works of art indicate what the conformation of these original animals was like and particularly whether they were humped or straight-backed. Could photographs of any such early and later works of art in which cattle are depicted be supplied?

3. If the straight-backed and humped types both existed in Egypt in the early ages, which was the original type; and, if the straight-backed was the original (which seems likely) when was the humped type introduced, from where, and by whom?

4. Which types of breeds predominate in Egypt to-day? Which is considered the best type or breed for beef and/or milk and why?

Morocco and Algeria.

1. Is the predominating type humped or straight backed?

2. Can the origin or breed history of the predominating types be given?

3. Can any photographs be supplied of rock drawings or other works of art depicting cattle of the early ages with approximate dates?

Nigeria (Northern and Southern) and Cameroons (British and French).

1. The closest possible description of the cattle of this area is desired with the breed history or origin particularly of two types, i.e. the Cow Fulani cattle, which are of the usual Zebu type (white with black points and humped) and the small type found in the forest country believed to be straight backed.

Belgian Congo.

1. A full description, the breed history, and origin of the large Ruanda cattle is greatly desired. The colour and markings, whether they are heavily humped, and the type of horn growth, beef, and milk qualities should be specially commented on.

Particulars of any other special types are also desired and particularly whether the small forest type mentioned under the Nigerias and the British and French Cameroons exists and, if so, where and to what extent and whether straight backed.

Norway and Sweden.

1. It is understood that the Scandinavian Fjällras or mountain breed are polled cattle mostly pure white with black points though, in many cases, silvering is noticeable. Is this correct?

2. Is it correct that they are entirely straight backed, i.e. without humps?

3. Any information as to their breed, history and origin, milking, beef qualities, hardihood, etc., will be greatly appreciated.

Brazil and the Argentine.

It is considered possible that the type of cattle in parts of Africa may have been influenced by introductions from Spain and Portugal during the period of Moorish occupation and particularly when the Moors were expelled from Spain; and, further south, they may have been introduced by Portuguese traders, after the discovery of the Cape. If this is the case, then it is likely that the type introduced would have resembled the type introduced into, and which still abounds in, many parts of the South American continent. It is therefore very desirable —

(*a*) To ascertain as nearly as possible the date when cattle were first introduced into South America.

(*b*) By whom were they introduced? Presumably the priests of that time?

(*c*) Were the animals introduced of any distinct type or breed recognised in Spain or Portugal to-day?

(*d*) Does the present Creollo type, descended from the original importations, closely resemble the original or foundation stock?

(*e*) An accurate description of the existing type, with photographs, of typical bulls and cows is greatly desired."

To summarise, it may be stated that:—

1. General information was required concerning the cattle of twelve African territories and five extra-African countries(¹⁵).

2. Special information was requested from six of the African territories and from each of the extra-African territories.

3. In regard to general information particulars were required with reference to:—

(*a*) Not only the country of origin but also the racial origin. Emphasis was laid on the evidence afforded by "paintings, rock drawings and sculptures, etc."

(*b*) The types of breeds recognised in each country, with details of the horns and humps.

(*c*) The value of the various types in regard to beef or milk production.

(*d*) The resistance of cattle to disease and adverse climatic conditions.

(¹⁵) It was possible to add information from other territories. These are indicated by asterisks.

(*e*) Special mention was made of colour, particularly white cattle with dark points, it being believed that this would throw light on the question of relationship of types.

(*f*) The territorial distribution of the various types.

(*g*) The associated environmental features, *e.g.* altitude, rainfall, type of vegetation, etc.

SUMMARY.

Apart from the Introduction, reference has been made:—

(*a*) To the steps leading to the present investigation;

(*b*) to the probable migration routes *in* Africa since the earliest folk-wanderings where such have been accompanied by cattle. As Prof. Dart (1933) remarks, " There is no more vital aspect of anthropology than the study of domestic animals ". Map I indicates both the probable migration routes *to* Africa and *within* Africa, along with suggested dates of these events;

(*c*) to a provisional classification of native cattle based on the presence or absence of the hump and then on the shape of the skull. Of interest is the fact that the so-called True Zebus are Asiatic in origin while the Pseudo-Zebus are African in origin;

(*d*) to the Questionaire issued to the governments of certain countries outside the Union of South Africa(16).

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(16) An important factor from the point of view of limitation is Glossina. Curson and Neitz hope to publish such a map shortly.

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CHAPTER II.

THE NATIVE CATTLE OF AFRICA. (17)

INTRODUCTION.

Further to the Questionaire it will be observed that in the data which follow, emphasis has been laid upon cattle types and their general distribution. The replies as a rule having furnished so little information in regard to environmental conditions, *e.g.* altitude, rainfall, and vegetation, it is felt, that having mapped out the distribution of cattle (see Map II), one could more profitably compare this with good topographical maps such as those already compiled by Shantz and Marbut (1923).

The same remark applies in general to other points raised, *e.g.* economic uses of cattle, resistance to disease and adverse conditions, maturity, etc. So little work has been done on beef and milk production that the authorities with justice could not be expected to reply in detail to this question. Where information exists it has been included in the text.

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(17) According to *Vet. Jl. of* 18.4.36 (quoting *Meat Trades Jl.*) the distribution of cattle by continents is Asia 185,541,000, Europe 141,984,000, South America 104,840,000, North America 93,830,000, Africa 22,547,000 and Australasia 18,606,000.

Concerning resistance against disease and adverse conditions in general, it can be accepted that native cattle, which have been subjected to the vicissitudes of the country of their origin for centuries, are able to withstand such adverse circumstances better than European cattle. Again local cattle are more resistant than introduced cattle. The term " acclimatised " expresses the state when animals introduced into a certain locality have acquired and are able to resist the many vicissitudes of the area in question, *e.g.* climatic conditions, pasturage and local diseases. A striking example of adaptation is the Shorthorned Zebu which according to the Tanganyika veterinary reports is superior to the Ankole.

In regard to maturity, while puberty is as early or earlier when compared with European breeds, production maturity cannot be expected as soon in Africa owing to the different environment.

(A) First and Second Migrations.

In a description of the cattle of North and West Africa we must consider the human migrations into Egypt, the gateway of Africa from Asia. As has already been described the early wanderings were along the Mediterranean shore to Morocco, and then either north into Europe or south to the countries around the Gulf of Guinea. We may now pass on to a description of the cattle in the territories associated with those early migrations.

The reply furnished by the Veterinary Service of the Egyptian Ministry of Agriculture and forwarded through the High Commissioner, Cairo, to the Department of External Affairs, Pretoria (High Commissioner's letter 248/6/32 of 4.4.32), states(¹⁸) as follows:—

Origin.

(*a*) " The date of cattle *domestication* is rather obscure, but it took place from the earliest stages of Egyptian history, even before the 3rd Egyptian Dynasty (*c.* 4000 B.C.). It is known that King Mina (Menes) was very interested in agriculture and land reclamation. He utilised both man power and cattle power to alter the position of the river Nile from the desert to its present position. Most of these indigenous animals were *polled* [our italics! Polled cattle were known in ancient Egypt—see Neffgen (1904) and Kronacher (1921)], but became extinct about 2000 B.C., and replaced by the types(¹⁹) mentioned " below. See Fig 4.

(¹⁸) Accompanying this were 18 excellent photographs, 4 of ancient monuments showing the Hamitic Longhorn type, 1 showing the original pre-Roman Brachyceros type (see Fig. 2 of " A Note on the Three Parent Stocks of African Cattle " by Curson, H. H., *Onderstepoort Jl. Vet. Sc. and Animal Indust.*, 1935), 2 of the present Shorthorned Zebu, 1 of the Afrikander, 2 of European cattle and finally 8 of the Egyptian cattle of to-day.

(¹⁹) Curson uses the term *type* to indicate the main classes of cattle, *e.g.* Hamitic Longhorn, Lateralhorned Zebu, Brachyceros, Shorthorned Zebu, Lyrehorned Zebu and Sanga. The word *subtype* is employed for each sub-class within the main class, *e.g.* in the Sanga main group are, among others, the following subtypes:—Ankole, Baila, Bechuana, Ambo, Zulu and Makalanga. *Breeds* may be evolved from the subtypes, *e.g.* Black " Nkone " and " Nyoniaipumuli " from the Zulu.

Photographs were supplied illustrating from ancient monuments the extinct type; but while these indicate horned cattle (Hamitic Longhorn), the text again refers to the cattle as being " mostly polled "! It is, however, considered that the term *horned* and not *polled* is meant. See Fig. 1.

(*b*) In describing the types of cattle *introduced into Egypt subsequently*, the following are referred to:—

(i) Afrikander. These " were brought into the land during the many . . . conquests of the ancient Egyptians . . . and therefore it is the oldest in date . . . nearly 3000 B.C. ". See Fig. 3.

(ii) European cattle (*Bos taurus*). These " were imported especially by the Romans on their conquest of Egypt(²⁰) and their settling to farming . . . ." See Fig. 4.

and (iii) The Zebu *i.e.* Shorthorned Zebu (*Bos indicus*). These were " imported especially by the Arabs during their invasion of the country and therefore it is of more recent date " (669 A.D.).

The result of the intermingling which has since followed is shown in Figures 5–12; but a comment made in describing the present cattle of Egypt namely that they " can hardly be considered now as distinct breeds " is significant.

(*c*) The above details summarised indicate that Egyptian cattle first became domesticated " probably before and during the Neolithic era " (Curson and Epstein 1934), but that about 2000 B.C. they became extinct. They were, however, replaced from without Egypt during three series of invasions, when (*a*) the Afrikander (presumbaly from Asia), (*b*) *Bos taurus* from Europe and (*c*) *Bos indicus* from Asia were introduced.

Present Cattle.

In view of the remark made above regarding the non-distinctiveness of the so-called types of cattle, it is perhaps advisable to refer to them not so much according to their conformation (the usual method), but in accordance with their geographical distribution. The document sent from Cairo gives the following information:—

(*a*) In *Lower Egypt* are to be found the (i) Damietta (Fig. 5 and 6) and (ii) Baladi or Beheri (Fig. 7 and 8) cattle, the former predominating along the coast.

(*b*) In *Upper Egypt* along the Nile occurs the Saiidi type (Figs. 9 and 10.)

and (*c*) In the *desert* are the Marriouti or Arabian cattle (Figs. 11 and 12).(²¹).

(²⁰) Octavianus (Augustus) defeated Cleopatra's forces at Actium in 31 B.C. and thereupon became master of Egypt. The Roman garrison consisted of one legion, approximately 4,000 men.

(²¹) According to information kindly furnished by the Chief Veterinary Officer, Palestine (his V/8/2/20 of 8.3.35), an Arab breed occurs in his territory. No photograph accompanied his description of the breed, but it is likely to be essentially of Brachyceros type as is the Marriouti beast.

" All types mentioned show predominance of red (of lighter or darker shade), occasionally black is found and also white spottings . . . the horns are nearly cylindrical, not longer than eight inches, bluntly ended with either horizontal outward or downward sweeps. The Damietta cattle have finer and occasionally more pointed horns with an upward sweep ".

" All types of Egyptian cattle are somewhat slow to mature, hardy against heat and drought (especially the Marriouti) and possess great resistance to indigenous diseases ".

" Dark points are met with in all types of Egyptian cattle, especially the black muzzle, eye rings, eye lashes, black hoofs and black legs under knees and hocks. Black skins covered with white hair are not found among Egyptian cattle . . . . Red animals with black shoulders, necks, and quarters are much in favour ".

Special features of the various types may be mentioned thus:—

(i) The Damietta cattle " are very slightly humped (due to the predominance of European blood) . . . and are valuable in their native district for milk purposes ". The distribution is, " predominates near the coast of the Mediterranean to the north-east of the Delta ".

(ii) " The Baladi type is the most valuable, because of its size and strength for work purposes. It is found in Lower and Central Egypt ".

(iii) Saiidi cattle are " met with in the southern provinces of Upper Egypt along the Nile banks ".

and (iv) The Marriouti type is " rare in numbers " and is to be " seen in the desert near the coast of the Mediterranean to the north-west of the Delta ".

In regard to immunity against local diseases, it is stated that " all Egyptian cattle are highly resistant to Stiff-sickness and Foot and Mouth Disease (not the suckling calves). They are also resistant to Texas or Egyptian Fever (except in some cases of debility and old age) ".

To summarise, it may be stated that while the conformation is somewhat similar in all Egyptian cattle (due to crossing " for very many generations "), yet geographically, certain groups are recognised, *viz.* the Damietta and Baladi in Lower Egypt, the Saiidi in Upper Egypt, and the Marriouti or Arabian cattle of the desert. These cattle possess immunity to certain local diseases.

Discussion.

Origin.—As indicated, *domestication* occurred " probably before and during the Neolithic era ", and the cattle were mainly of long-horned and humpless type, now called the Hamitic or Egyptian Longhorn. It is not agreed by the authors (H. H. C. and R. W. T.) that these cattle became " extinct about 2000 B.C."

As to the *subsequent introduction* of cattle, whereas Curson and Epstein believe that they arrived in the order Brachyceros, Lateral-horned Zebu (Afrikander) and Shorthorned Zebu, the Egyptian authorities hold that the Afrikander came first, to be followed by European (? Brachyceros) cattle during the Roman occupation and then by the Shorthorned Zebu. It is generally accepted that the last named accompanied the expansion of Islam during the VIIth century.

The cattle stated to be of European origin are of Brachyceros type, and are believed by Curson and Epstein to have been introduced *prior* to the Roman occupation *from Asia*, as the " mural reliefs of the temple of Hatschepsut in Dair-al-Bahri " (Yahuda 1934, p. 8) show. Their influence on the present cattle of Egypt is striking.

Epstein (1933) has referred to the part played by the Lateral-horned Zebu in Egypt, and according to him the Sanga (or so-called native cattle of Central and Southern Africa) represent the cross between this Zebu and the original Hamitic cattle. Judging by the hump of the Egyptian cattle of to-day, the importation of Lateral-horned Zebus (Afrikander) into North-East Africa must have been on a very large scale(22).

Present Types.—Confirming the observation that there is a uniformity of Egyptian cattle, owing to the intermingling of centuries, is Flower's (1932) comment that in his time (?) " the domestic *cattle* of Egypt . . . were all of one shorthorned type from Alexandria to Aswan ".

One naturally enquires what the photographs indicate? The reply is that with the exception of the hump which is *cervico-thoracic* and best marked in the bulls, the cattle show Brachyceros features. The hump, which is also prominent in the Saiidi cow, Fig. 10, is of the shape observed in the Afrikander and Sanga types and *not* of the form and situation observed in the Shorthorned Zebu, which according to the opinion of the Ministry of Agriculture, Cairo, is responsible for the marked hump of the Saiidi cattle.

What has probably happened is that formerly (up to some centuries ago), the cattle of Egypt were generally of the Sanga type; but that through the constant introduction of cattle of the Brachyceros type (now admittedly from Europe), the conformation has become almost Brachyceros-like, the hump, however, retaining the characteristics of that of the Afrikander (or Sanga) type. In this connection it is significant that the " Damietta cattle *are very* slightly humped " (due to the predominance of what Curson and Epstein prefer to term Brachyceros(23) " blood ".

As will be manifest later, in Algeria apparently all evidence of the cervico-thoracic hump has disappeared and the cattle are definitely of Brachyceros type.

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(22) On the other hand the importation and influence of Sanga cattle from the Sudan must also be considered.

(23) The Cairo reply uses the word European.

It would seem that in Egypt unlike in East Africa, the Shorthorned Zebu did not swamp the country(²⁴). Again the same process, as described in the last paragraph but one, is occurring in the Subcontinent to-day, where cattle of Sanga type through indiscriminate crossing with European cattle are being replaced by nondescript animals of Brachyceros type.

French North Africa.

The subjoined particulars were provided mainly by the Heads of the Service de l'Elevage of Algeria and Morocco, who were approached in the first instance through the British Embassy, Paris. The particulars forwarded from Paris to Pretoria were sent under cover of letters dated 25th January, 1932 (concerning Morocco) and 9th March, 1932 (concerning Algeria) and addressed to the Minister of External Affairs (General The Honourable J. B. M. Hertzog).

Origin.

The cattle of North Africa are essentially of one type, Brachyceros; but through differing environment and admixture in the distant past the blood of other types (*e.g.* Afrikander in Egypt and probably Hamitic Longhorn in North West Africa), sub-types have arisen. From these sub-types, breeds have been created chiefly by selection, on the initiative of the French authorities, when they took possession of the country. Johnston (1906, p. 908) refers to rock engravings of cattle in the Sahara.

While the relationship with the Brachyceros cattle of Europe is generally recognised, Saint-Hilaire is not inclined (p. 189) to support the theory of an origin from Asia by way of Egypt.

Present Cattle.

The territories to be considered now are Tunis, Algeria and Morocco.

*Tunis.**

No questionaire was sent to Tunis, but for completeness information bearing on the country will be given.

According to Saint Hilaire (1919), the cattle resemble those of the Guelma " variété " of Algeria to which we shall refer later. He adds that the cattle of Tunis are among those having the best conformation in North Africa, owing to improvement brought about by local selection and thus giving the most rapid results (p. 194). See Figs. 13 and 14 (Plate XIX of Saint-Hilaire 1919) which show a typical Tunisian bull and cow.

Algeria.

In addition to Saint-Hilaire's volume, replies to the questionaire are available from the Departments of Constantine, Algiers and Oran. Unfortunately not the originals but their translations were received for this compilation.

(²⁴) Little or nothing is known of the genetics of the hump. It is obviously of importance in classification.

The following breeds (variétés) are to be found:—

(*a*) Cheurfa occurring in the Department of Constantine.

(*b*) Guelma found throughout the country, but chiefly in the eastern part of Algeria.

(*c*) Kabyle from Constantine to Algiers.

(*d*) Oran found in the Department of the same name.

And (*e*) Chaouia occurring in the Department of Constantine. This breed is mentioned in the reply from Constantine, but no particulars are given except that it resembles the Guelma and Cheurfa breeds.

Illustrations are available of the Chuerfa bull (Fig. 15) and of the Guelma bull and cow (Figs. 16 and 17).

The Cheurfa breed is believed by some authorities to represent the " variété " from which the Guelma was evolved (Saint-Hilaire, p. 192), since its distribution, especially in the south of Constantine, is more extensive than that of the Guelma. The main differences are the better development of body and the " prédominance de blanc " of the coat, giving a light grey colour. " The coat of the bull is always darker than that of the ox and the cow " (Reply of 25.4.32 from Constantine). In general the Chuerfa is taller and bigger and more in demand than the Guelma.

It is clear from the reply from Algiers and Oran that the authorities do not distinguish between the above two breeds, for the cattle of Algeria are roughly classed into (*a*) the Guelma of eastern Algeria and (*b*) the Moroccan cattle of western Algeria. The former are described as short, the height varying from 1·15 m. to 1·25 m., the horns are small and carried horizontally, they are sharp pointed and the colour is white at the base and black at the tip. There is a band of white hair above the muzzle which with the buccal mucosa is black. The colour of the coat varies from a deep iron grey of the head, neck, shoulders, lower flank, and leg to a pale grey elsewhere, except the under surface of the belly which in the majority of cases is white. The conformation is pleasing from an European standpoint, the body being thick set and the ribs well rounded. As is noticeable in Figs. 16 and 17, the tail is long, light grey in colour and ends in a black tuft.

The Kabyle breed is an excellent example of the effect of environment on conformation. Through grazing on poor pastures for generations the cattle, which are of Brachyceros type, have degenerated and vary now in size from 0·8m. to 1·15m. While in general resembling other cattle of East Algeria, the Kabyle has in proportion a longer skull and is " plus osseuse que le Guelma " (Saint-Hilaire, p. 192).

The Oran cattle are described as somewhat less regular in build than other Algerian cattle, but bulkier and larger in size, varying between 1·20 m. and 1·25 m. They have no doubt been influenced by Moroccan cattle which appear to possess some proportion of Hamitic Longhorn blood.

Morocco.

The Chief of the Cattle Breeding Service of Morocco writing from Rabat on 29.12.31 states that the origin of Moroccan cattle is uncertain.

Moroccan cattle occur also in the Department of Oran, " being met with regularly in the markets of Marnia and Ain-Témouchent ". They are bigger than the eastern cattle and range up to 1·40 m. in height. The horns grow outwards and upwards in a regular curve . . . the withers are thick and well defined, the back broad and its line often regular. The rump tends to narrowness with the haunches protruding. . . . The fore limbs are strong and regular and the chest deep. . . . The coat varies from jet black to dull white, passing through all shades of red which is the predominating colour (Reply from Oran). See Figs. 18 and 19 illustrating the type (Plate XXI from Saint-Hilaire).

Saint Hilaire states (p. 196) that in East Morocco the cattle of the mountainous region are short with a dark coat and resemble the Kabyle cattle.

In a publication issued by the Service del'Elevage in 1923, while it is stated that the cattle are characterised by a lack of uniformity, two breeds, however, may be recognised. The first, *la race brunc*, has a dun coat with black extremities and the second, *la race blond*, has a pale coat with the extremities slightly pigmented or without pigmentation at all.

Attempts at improving the native herds by grading up with European, especially French breeds, and the Shorthorned Zebu, are being made.

North African cattle are stated to be indifferent milkers, especially those of Western Algeria. The reply from Constantine describes the milk as rich in butter-fat, and that from Algiers and Oran gives the average milk yield as up to six litres, although 8-10 litres may be occasionally obtained. Saint-Hilaire believes that in Morocco with its better climatic conditions the possibilities for improving the milk yield are superior to those of Algeria and Tunis. He adds that in the " Vallée du Sebou par exemple, elles donnent jusqu'à 14 à 15 litres " (p. 196).

In regard to live weight he states (p. 195) that whereas the average figure for Algerian and Tunisian cattle does not exceed 250 Kg. probably 50 Kg. less according to the Oran data), the cattle of Maghret, Morocco, give an average of 275 Kg. Cattle are, however, met with which exceed 400 Kg.

West Africa.

As will be seen (p. 12) questionaires were sent only to Nigeria and the French Cameroons (Mandated Territory), but it is felt that information bearing on other territories should be recorded, for it is in West Africa that least is available about indigenous cattle.

West Africa represents, as does South Africa, a *cul-de-sac* and it is the furthest point which could be traversed by tribes migrating from the north and east. Unlike South Africa, however, the unfortunate nomads could not continue following the coast-line southwards (as did the Hottentots in South Africa) for their advance was blocked by the immense equatorial barrier of Glossina. Presumably what happened was that the earliest people with their Hamitic cattle did not penetrate the dense forest region bordering the Gulf of Guinea, but dispersed in the more open country between French Senegal and Northern Nigeria.

The succeeding wave of migration, with Brachyceros herds, on finding only the littoral unoccupied were accordingly compelled to inhabit the Glossina infested jungle along the Gulf of Guinea. These territories are known to-day as French Guinea, Liberia(25), Ivory Coast, Gold Coast, Dahomey, Southern Nigeria and French Cameroons. Through living in such an unfavourable environment Brachyceros cattle have in the course of centuries (or rather millenia) deteriorated and are now considerably smaller in size than their relatives in Europe, *e.g.* the Jersey, Guernsey and Kerry. While dwarf-like and of no value at present for dairy purposes, the type is at least resistant to Nagana, a malady which would kill European cattle in a few weeks.

Above have been outlined the First and Second Migrations, but a description of West African cattle would be incomplete, unless reference were made to the Fifth and Sixth Migrations. The former concerns the arrival in West Africa, in the vicinity of Lake Chad, of nomads from North-East Africa (probably Abyssinia), accompanied by Sanga cattle. As will be seen in Fig. 36, these cattle, represented to-day by the Bornu cattle, are similar to the Sanga cattle of South and Central Africa.

The Sixth Migration refers to the passage of the Shorthorned Zebu from the East African coastlands westwards north of the Equatorial " fly " region, to Northern Nigeria and French West Africa (north of the Glossina region). From these cattle, along with the early Hamitic Longhorn stock, has arisen the Lyre-horned Zebu, best represented in West Africa and to which this is the first reference as a distinct type. Stewart of the Gold Coast writes (14.1.36) that the cross between the Shorthorned Zebu and Brachyceros has given rise to an intermixture which exists in large numbers, but until more details are available as to the uniformity and conformation of the hybrid, it cannot be considered in a general survey. It was Pierre (1906) who attributed the origin of what is apparently the Sanga (see Figs. 24-26) to this cross.

Another new feature is the use of the term Lateral-horned Zebu (in place of longhorned Zebu) for Afrikander, made necessary by the incorporation of a sixth cattle type, the Lyre-horned Zebu which is also longhorned.

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(25) On Sir Harry Johnston's evidence Hamitic Longhorn cattle also occur in Liberia.

To summarise, it may be stated that the following cattle types are to be found in West Africa:—Hamitic Longhorn (few in Liberia), Brachyceros, Shorthorned Zebu, Sanga, and Lyre-horned Zebu.

Each territory will now be considered separately.

*French West Africa.**

It was from a French veterinarian, Pierre, that the first description of West African cattle was received. In his *L'Élevage dans l'Afrique Occidentale Française* Pierre classifies the genus Bos thus:

(*a*) " les bovidés zébus " *i.e.* humped cattle, and

(*b*) " les bovidés taurins " *i.e.* non-humped cattle.

(*a*) The term zebu in West Africa, as indicated above, appears to include not only the Shorthorned Zebu seen in East Africa, but also cattle with lyre-shaped horns and a thoracic hump. The general information suggests a strong infusion of Hamitic Longhorn blood, which can be well understood when one considers Sir Harry Johnston's (1906) evidence concerning Mandingo cattle in Liberia.

The Zebu is described as occurring throughout French West Africa north of latitude 14°. South of this line trypanosomiasis and piroplasmosis are the factors limiting its extension.

Pierre, as the result chiefly of environmental influence, further groups the zebu as follows:—

(i) The Peulhe or Gobra sub-type,(26)

(ii) The Moorish or Gabaruyé sub-type,

(iii) The Nigerian or Foulbé sub-type, and

(iv) The Fogha sub-type.

The four sub-types are shown in Figs. 20-23. The Peulhe and Moorish cattle quite evidently belong to the Lyre-horned Zebu type. It must be remembered that in British Nigeria several types of Zebu cattle also occur, and these are not to be confused with the French Nigerian Zebu.

(b) Pierre includes in the designation taurine, not only the Ndama cattle (of Brachyceros type), but also the intermixtures arising between cattle of Zebu and Brachyceros types. In this paragraph reference is made only to true Brachyceros cattle, their crosses being left to the following paragraph. Pierre mentions the Race de Borgou and Race Cotière, but these are also " *varieties* " of Brachyceros(27).

Among the derived types or cattle originating through crossing are:—(*a*) The Bambara or Mandé and (*b*) the Djakoré or Sengalese cattle. The *former* is described by Pierre as uniform and widely distributed. Figs. 24 and 25 show the conformation. It will be noted that there is a fairly prominent cervico-thoracic elevation, and Pierre, instead of describing a " bosse " or hump, mentions that the withers (" le garrot ") are sometimes elevated and very large. The *latter* is said to be the cross between the Gobra bull (Lyre-horned Zebu) and the Ndama cow (Brachyceros). The hump (*i.e.* the thoracic musculo-fatty structure) is said to have disappeared, but remarkable to relate, the Djakoré beast " reprend tous les caractères zootechniques du père à la 3e ou 4e génération " (p. 109). See Fig. 26.

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(26) Pierre actually uses the word " variété ".

(27) Pierre describes the Race Cotière as frequently being hornless. Stewart in the Gold Coast encountered six polled cattle out of approximately 200,000 (private communication).

Pierre makes no reference to Mandingo cattle which Johnston describes as " the dominant type of the Mandingo Plateau and the regions of the Upper Niger, Senegal . . . " (p. 907). See Figs. 27 and 28.

*Gold Coast.**

Thanks to Pierre and Stewart (P.V.O., Gold Coast) much information is available concerning Brachyceros cattle not only in the Gold Coast but throughout West Africa. It was the second authority who first used the name West African Shorthorn for cattle of this type. Two papers (by Epstein and Curson respectively), summarising the chief facts regarding Brachyceros cattle in Northern Africa, were published in the *Journal of the South African Veterinary Medical Association*, Vol. V, No. 3, 1934.

Stewart (*Ann. Rpt.* 1929-30, p. 10) confirms Pierre's observation that the country south of latitude 14° is not occupied by Zebu cattle in any number. He states that " these cattle (zebu) are imported (from French territory) and travel to the markets of the Colony and Ashanti for slaughter." He refers to the importation of zebu bulls for breeding purposes and adds (p. 17) that " the best type of zebu for crossing is the short deep chested beast which is bred in the north-west of French Haute Volta." Presumably the bull he shows opposite p. 17 is of good type. It resembles somewhat the East African Shorthorned Zebu except for the horns which suggest Brachyceros influence. See Fig. 29.

Referring to the cross between the Zebu bull and Brachyceros cow Stewart gives an excellent photograph of the calf which is humped, a feature contrary to Pierre's experience. See Fig 30.

Stewart in discussing resistance to disease states that Brachyceros cattle are more resistant to Nagana, Lungsickness and Streptothricosis(28) than the Zebu, while the latter are more resistant to Rinderpest. This resistance to Nagana is transmitted to the progeny.

According to the same authority (*Annual Report*, 1934-35) there are 192,000 cattle in the country.

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(28) In the *Annual Report Vet. Dept. Nigeria* for 1925 (p. 15), this is called Contagious Impetigo. It is not clear whether this is the Contagious Impetigo described by Hornby in *Vet. Jl.*, June, 1920, in Central Africa. See also a note on Saria by Curson in *Vet. Jl.* of Nov., 1920.

*Liberia.**

As far back as 1906 Johnston described " the black and white or brown and white dwarf small horned cattle " as " the dominant breed in the coast regions of Liberia " (p. 907). He adds " the other type of Liberian cattle, generally called the Mandingo ox, is an interesting form, which in some respects suggests a dwarf variety of the Egyptian longhorned, straight-backed, uniform-coloured cattle ". He believes the Mandingo to be " a hybrid between the *Bos taurus*(²⁹) and *Bos aegyptiacus* " which view appears very probable, the Hamitic characteristics predominating except possibly for size.

Johnston, in a footnote (p. 908), states that rock engravings in the Sahara indicate the use of cattle as transport animals " before the camel became abundant." Possibly close inspection of the engravings would show the type of cattle employed *e.g*,. whether Hamitic, Brachyceros, Shorthorned Zebu or Sanga! See Figs. 27 and 28.

*Sierra Leone.**

Johnston (1906, p. 907) describes the Mandingo beast as " the dominant type . . . of Sierra Leone ", but the Brachyceros is also present (Stewart *Ann. Rpt.* 1930-31).

According to Minute A/76/29 of 28.5.36 from the Colonial Secretary, Sierra Leone " The Acting Director of Agriculture reports that cattle in Sierra Leone are all of the same breed—the Mandingo ox—". Fig. 1, Plate XVIII of Dr. J. J. Simpson's article on Entomological Research in Sierra Leone (*Bull. Entom. Res. IV* 1914) shows cattle resembling a Hamitic Longhorn-Brachyceros cross as indicated by Johnston. Only examination of the skull, however, will solve the point. The Brachyceros-Shorthorned Zebu and Brachyceros-Hamitic Longhorn crosses urgently require study.

*Gambia.**

Although nothing definite is known, it would seem that *Bos brachyceros* is the dominant type of Gambia. In any case the cattle population is very small (letter 1184/30/1911 of 16.10.35, P.V.O., Gold Coast).

*Portuguese Guinea.**

The cattle are essentially of Brachyceros type and according to Da Costa (1931) comprise two distinct sub-types (" races "), *viz.*: the Foulah which has a straight profile of the head, is white in colour, and found along the Oco; and the Manjaca, occupying the Brames region, with a concave profile and black coat.

The cattle population in 1932 was 62,000.

Nigeria.

The Questionaire was answered by Capt. W. W. Henderson. M.R.C.V.S. and the reply sent through the Chief Secretary's Office, Lagos (12636/62 of 11.3.32) to the Secretary for External Affairs.

(²⁹) Johnston's name for *Bos brachyceros.*

In a former paper Curson (1934) endeavoured to classify the cattle of this Colony according to the information supplied by Brandt (1925); but as suggested on that occasion recent additional evidence now makes a revision of the classification necessary. Brandt's article was unaccompanied by illustrations, but apparently the position is that " with the exception of the small percentage of humpless cattle(³⁰) owned by the Pagans the cattle are all of the Zebu type " (Anderson, A.W. 1933). The type of beast, in most cases, resembles the Lyre-horned Zebu of French West Africa. See Figs. 31-34.

As far as sub-types are concerned, the following is Brandt's original description:—

(*a*) The Fulani, " a large breed with the prevailing colour white, although some are found with small black flecks. The hump is large and the horns are long and curved upwards." See Figs. 31 and 32(³¹).

(*b*) A smaller edition of (*a*).

(*c*) Another Fulani breed, being ". large-humped, narrow bodied, red . . . with long wide-spreading horns." See Fig. 33.

(*d*) " A long-backed humpless breed with medium length horns of huge diameter; the prevailing colour is white, but all colours are met with; found chiefly in Bornu." This is the Sanga(³²).

(*e*) " The Shuwa of Bornu—a parti-coloured, medium sized, very compact breed, with short legs, practically no hump and short horns."

From recent issues of the *Annual Report of the Agricultural Department, Nigeria*, it is learned (*Report of* 1930) that herds had been established of the (*a*) White Fulani, (*b*) Shuwa-Bornu and (*c*) Adar-Sokoto-cattle, and later (*Report of* 1933) the Godali-Sokoto-cattle are mentioned. Particulars with regard to conformation and utility are, however, not yet available, but Figs. 31-36 show the type.

It is gratifying that investigational work affecting native cattle is being undertaken in Nigeria, an important contribution being that of Anderson (1933) who summaries his observations as follows:—

> " 1. Nigerian pastures are of low feeding value, especially during the dry season. As judged by chemical standards the mineral balance does not appear to be abnormal but pica is prevalent and is prevented or cured by administration of sodium. Increasing the caloric, but not the mineral intake, improves rate of weight and hastens sexual maturity.

(³⁰) *i.e.* Brachyceros.

(³¹) It is not stated what type of hump occurs. It appears to be a Lyre-horned Zebu. See Curson and Bisschop (1935).

(³²) The hump is cervico-thoracic and small. See Fig. 36.

> 2. The standard of the cattle is very low in spite of careful selective breeding on the part of herd owners. Environmental conditions are such that *improvement by European stock is impossible.* With the maximum improvement in nutritional conditions economically possible, the most advantageous policy appears to be to breed within the existing herds for a combination of high milk yield and high reproductive rate."

One is interested in the remark that "careful selective breeding" is undertaken by herd owners. Nigeria must be one of the few parts of Africa where this is done by natives.

Anderson's view supports that of du Toit (1927) who, in discussing cattle improvement in Nigeria, recommended "selection from native stock" in preference to grading up with European stock.

French Equatorial Africa.

French Cameroons(33).

The only information relating to Equatorial Africa is that concerning the French Mandated Territory of the Cameroun. The reply to the Questionaire was arranged through the British Embassy, Paris, which forwarded the reply in question to the Minister of External Affairs under cover of a letter dated 26th April, 1932(34).

The reply is instructive not only for the description of cattle types but also for information on cattle husbandry.

Cattle Types.

The cattle are either non-humped or humped(35). The former group comprises Brachyceros, this being almost if not, the extreme southern limit of distribution. The cattle are said to be ·75–1·m in height, very well built and very lively. Strangely they live in the mountains, but this is due to the fact that their owners were forced to retreat from the fertile plains of the Diamaré and the Adamaona at the time of the Peulh migration about 25 years ago. "The small animal, which is on the way to extinction still exists in small herds in the Mandara, in Namchi-Allan-Tikas and in the south. It does not constitute a commercial article and the kirdi or pagans rarely concern themselves with it, for they do not drink milk and only eat beef in exceptional circumstances. On the death of a pagan one or more oxen are sacrificed and the corpse is rolled up in the hides of the slaughtered animals."

The latter group is of recent origin and appears to include the following types:—(*a*) Shorthorned Zebu or Arabian humped cattle, (*b*) Lyre-horned Zebu and (*c*) Sanga.

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(33) No reply can be traced from British Cameroons, now a part of Nigeria.

(34) Unfortunately the French version was not available, a translation having been made before reaching me. (H. H. C.)

(35) The reply states that the cattle of the river region, called Kouri after the tribe owning them, are non-humped; but the description indicates they are of Sanga type, being similar to the Bornu cattle of Nigeria.

The Arabian cattle or Choas are described as " well assorted, well formed " and intermediate in height between the next two sub-types to be described, *viz.*: Poulfoulo and Mbororo—1·30-1·40 m.—" They fatten easily and are widely used for the transport of women and merchandise. Their home is in the districts of Fort Foureau and Mandara and from Mandara onwards to Lagone in Upper Balgué ".

The Lyre-horned Zebu appears to be represented by the short Poulfoulo (1·25-1·35 m.) and the tall Mbororo (1·3-1·7 m.) cattle. " The Poulfoulo sub-type is owned by the Peulhs or Poulhos who arrived in two distinct migration waves, one *via* Baguirmi and the other *via* Northern Nigeria. The cattle are prolific and hardy and survive everywhere. The skeleton is small and light, but the capacity to put on beef is marked, the weight of four quarters (minus the head) varying between 55-60 per cent. in the case of adult oxen. The milk yield is low, 3-4 litres a day being the average. Good milk cows with sufficient feeding may give up to 6 litres. The milk (as is generally the case in humped cattle) is rich in fat, 19 litres yielding 1 Kg. of fat ".

The Mbororo cattle, usually dark chestnut in colour, are owned by the Mbororo people who are essentially nomads and related to the Peulhs, who, however, are settling down in small colonies or villages under a *lamido* (chief or sheik). The cattle are characterised by immense upward curving horns, and a powerful frame. They would be suitable for draught purposes but for slaughter are inferior to the Poulfoulo.

The Sanga or Kouri cattle are of large size (1·4-1·5 m.) and possess horns of extraordinary length and diameter, which nevertheless are remarkably light. The coat is generally light in colour (ivory) and the cattle found about Lake Chad are wonderful swimmers, travelling from island to island under the direction of the herd boy.

Cattle Trade.

Although attached to his herd, the breeder has from time to time to dispose of a certain number of animals in order to provide for his needs. These are either slaughtered in the large centres, especially on market days, or driven to the south of the colony where stock-breeding is impossible, or lastly, and this is the most frequent, to Nigeria. Generally old cows and those which only calve very rarely, infertile heifers, old reproducers and superfluous bull-calves form the subject of these transactions. Fertile cows are only sold on the market in exceptional circumstances and are generally exchanged for other stock, mostly horses. The young bulls are taken to the market at the age of 2, 3 or 4 years. Sometimes the traders go to the villages and make their purchases from amongst the flock. If intended for Nigeria the animals bought on the various markets are grouped in herds; these by successive stages reach the big markets of Maidougari, Kano, Sorao, and are sometimes driven as far as Port Harcourt. The cattle trade with Nigeria is by far the most important, and consists mostly of animals coming from the weekly markets of Marona, Mindiff, Bogo Fudere and Adanuri.

The reasons for this trade movement towards Nigeria are as follows: —

A higher rate of exchange.

Traffic facilities, improved roads, and, generally speaking, safety from tsetseflies.

Facilities exist for exchanging the money obtained for goods (easily saleable at a profit on their return) such as fabrics, silks, pearls, sugar, salt, etc. These commodities are abundant at Maidougari and Kano.

Animals purchased in the north of the colony for 300 francs have fetched prices ranging from £4 to £5 at Yerona.

Apart from the private Compagnie Pastorale, the cattle trade is in the hands of the Hausas. A few Europeans installed at Marona have for some years been draining large numbers of cattle into Nigeria. At present they seem to prefer the trade in oxhides and sheepskins.

The cattle number approximately half a million, and are confined mostly to the northern districts.

The absence of photographs, while to be regretted, in that confirmation of the classification is not possible, is amply compensated by the detailed description provided by the French authorities.

SUMMARY.

In regard to the present cattle types and their distribution, this information, associated with the First and Second Migrations of Hamitic Longhorn and Brachyceros cattle respectively, is actually summarised pictorially in the photographs and in Map II. To discuss each country individually it may be stated as follows: —

Egypt.—(1) The view of the Egyptian authorities in regard to *origin*, is more or less in accord with that published independently by Curson and Epstein (1934). Certain points advanced by the Cairo reply requiring further consideration are: —(*a*) belief that original cattle of Egypt were polled(36) and became extinct about 2000 B.C.

(2) The monuments of Egypt furnish valuable evidence concerning the early history of the country, the types having been Hamitic Longhorn, Brachyceros, Lateral-Horned Zebu (Afrikander) and a polled type having the body conformation of the Hamitic Longhorn.

(3) The cattle of to-day are more or less uniform being best described as belonging to Brachyceros. The cervico-thoracic hump accompanying a Brachyceros conformation is remarkable.

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(36) It is quite likely that a typing error occurred either in Cairo or Pretoria, the word "horned" being meant. The compiler of this contribution (H. H. C.) did not see the original reply. See p. 21.

French North Africa.—(1) The cattle of French North Africa are of one dominant type, Brachyceros, and as one proceeds westwards to Morocco they are coarser due possibly to the influence of cattle of Hamitic Longhorn type, which persisted there longer than elsewhere.

(2) Environmental changes, chiefly reflected in the poor pasturage, are responsible for the evolution of several sub-types of Brachyceros cattle.

(3) A few details are given concerning Tunis, a territory not included when the original Questionaire was despatched.

West Africa. French West Africa.—(1) Cattle of the Brachyceros, Lyre-horned Zebu and Shorthorned Zebu (but not the Lateral-horned Zebu) types occur. Whether pure Hamitic Longhorn cattle still exist is unknown.

(2) In addition there is the Bambara which is said to be a hybrid (Zebu × Brachyceros) but from Figs. 24 and 25 it appears to resemble in conformation the Sanga. The Djakoré too is apparently also a representative of the same type.

Gold Coast.—(1) The dominant type in this territory is Brachyceros.

(2) Stewart notes the resistance of Brachyceros cattle to Nagana, Lungsickness and Streptothricosis and the resistance of the Zebu to Rinderpest.

Liberia.—(1) The dominant type in Liberia is also Brachyceros, but it appears probable that descendants of Hamitic Longhorn cattle still occur.

Sierra Leone.—(1) According to Johnston the Maudingo (or as it appears to be, a Hamitic Longhorn-Brachyceros cross) is the dominant type. Brachyceros is also probably represented.

Gambia.—(1) Apparently Brachyceros is the dominant type.

Portuguese Guinea.—(1) The Brachyceros cattle are of two main sub-types, Foulah and Manjaca.

Nigeria.—(1) The types represented are Brachyceros, Shorthorned Zebu, Lyre-horned Zebu and Sanga.

(2) Research work is being carried out in regard to nutrition and animal husbandry.

French Equatorial Africa. French Cameroons Mandated Territory.—(1) Not only do Brachyceros cattle occur, but also apparently (there being no photographs) Shorthorned Zebu, Lyre-horned Zebu and Sanga.

(2) The cattle trade is briefly referred to.

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(B) THE THIRD AND FOURTH MIGRATIONS.

In describing the cattle of Central and South Africa we must have in mind the Third and Fourth Migrations referred to previously and indicated on Map I. While it would seem that the Hottentots of the Third Migration travelled together on their southerly journey, the Bantu tribes of the Fourth Migration, on the other hand, showed a tendency either to migrate in separate bodies and to settle where conditions suited, or if the migration was *en masse* to hive off and occupy country which appealed to them, irrespective of the general southward direction taken by probably the most adventurous of the nomads.

The reason for this view is that the cattle of the Hottentots, *i.e.* Lateral-horned Zebus, are to be found nowhere else in Africa but in the Union of South Africa. Of course within recent years the Afrikander has been introduced into the northern territories, *e.g.* Northern and Southern Rhodesia, where centuries ago its ancestors roamed at will. The Sanga cattle of the Bantu tribes, on the other hand, are to be found scattered along the migration route from Abyssinia to the Sub-continent, *i.e.* in the vicinity of the Great Lakes, except Lake Nyassa, where the Shorthorned Zebu now predominates. For convenience the Sanga cattle of Central Africa are shown on Map I as Ankole but this term has also a restricted meaning.

(³⁷) Only new (*i.e.* not in previous list) references given.

It is apparent that the extension westwards from the East Coast by the Shorthorned Zebu is of comparatively recent date(³⁸). It seems likely that the Sanga was found throughout East Africa before the arrival of the Shorthorned Zebu, in fact, according to Epstein, it still occurs in Madagascar. Orde Browne (1925) in describing the Zebus of Kenya between Mt. Kenya and the Tana River, refers to cattle of the Sanga type in Kenya as recently as 1890 in his statement (p. 117), " There was, however, a breed of cattle which has now died out, which possessed far larger horns than the existing breed (Zebu), and are said to have been finer animals in every way. They are said to have been very numerous and to have been killed off in the epidemic of rinderpest, which occurred apparently about 1890. . . . There are no traces of these animals now left, except the horns, which are occasionally to be met with, made into drinking vessels; these nearly always came from Emberre ".

*Abyssinia.**

According to Volume 1 of the *Encyclopaedia Britannica* (14th Edit., 1929, p. 73) the number of cattle in Abyssinia is estimated at between 10-15 millions. As it is in that part of Africa where the Sanga cattle are believed to have originated, it is convenient to refer to the cattle of to-day, " of which the most remarkable are the immensely horned Sanga or Galla oxen ". Most cattle are of Shorthorned Zebu type [letter of 18.10.35 from Col. R. J. Stordy, M.R.C.V.S.(³⁹)], but according to the *Encyclopaedia Britannica* " there are also two breeds—one large, the other resembling the Jersey cattle—which are straight backed ". While it is evident that the cattle of Jersey type are Brachyceros, it seems likely that the large straight-backed cattle are in reality Sanga in which Hamitic Longhorn characteristics predominate, and the hump (cervico-thoracic) accordingly is not prominent.

The respective areas of distribution are roughly Brachyceros in the north, Shorthorned Zebu in the eastern districts and Sanga in the west.

*Anglo-Egyptian Sudan.**

The *Annual Report of the Veterinary Department for* 1925 (p. 16) groups the native cattle as follows:—(*a*) Shorthorned Zebus, probably throughout the eastern part of the territory, (*b*) Sanga along the Upper Nile to link up with cattle of the same type in Uganda and (*c*) a type along the Abyssinian boundary, apparently of Brachyceros origin.

(³⁸) Tissie (*Proceedings 5th Pan-African Veterinary Conference*, 1923, p. 116) believes that the zebu of Madagascar was " primarily imported from Africa " by the Arabs who " colonised the Comoro Islands during the 9th century ", and then went to Madagascar.

(³⁹). Col. Stordy was the first veterinarian of Kenya and once travelled through Abyssinia. Recently he accompanied a veterinary section to the latter country during the Italian campaign.

Uganda.

Information on the cattle of Uganda was supplied by the Acting Director of Veterinary Services (Hart, R. L. L.) and forwarded to the Secretary for External Affairs by the Chief Secretary, Entebbe, on 12th October, 1932.

On account of its preciseness it is subjoined hereunder.

" The cattle of the Uganda Protectorate are descended from two widely divergent types, the straight backed, long horned Nsagala or Ankole cattle of the Western Province and the humped Zebu cattle of the Eastern and Northern Provinces. Cross bred stocks intermediate between the two parent strains exist in these provinces and in Buganda.

2. The Ankole cattle are supposed to have been brought originally from the North, the Zebu from the East. It is impossible to give an approximate date as to when such introduction occurred, *vide* paragraph 7.

3. Ankole cattle are straight backed with a most rudimentary hump, the Zebu humped; crosses vary in accordance with the degree to which the respective strains predominate.

4.

ANKOLE AND ALLIED BREEDS OF CATTLE ALSO CROSSES PREDOMINANTLY ANKOLE.

Typical Colours.	*Luhima Name.*
(1) Deep red.	Bihogo.
(2) Red.	Gaju.
(3) Red and white.	Magabo.
(4) Light red.	Sina.
(5) Yellow.	Kisa.
(6) Strawberry roan.	Luhusimu.
(7) Red (white spots numerous).	Mayenje.
(8) White markings few.	Kiremba.
(9) Red (white blaze on face).	Kyasa.
Less Common Colours.	
(10) Black.	Kyozi.
(11) White.	Kitale.
(12) White (red spots).	Bugondo.
(13) Brindle.	Ngobe.
(14) White blaze on throat.	Kiroko (Toro).
(15) Black (red spots on back).	Kikara (W. Masaka).
(16) Grey and black.	Nsama (Toro).
(17) Red and black.	Njumba (Toro).
(18) Grey.	Mpula ; Mbubi.
(19) Black and white (definite recognised colour schemes, but difficult to describe.)	Mutambulire. Mpuga.
(20) Dark body, white head.	Ihinda.

ZEBU AND PREDOMINANTLY ZEBU STRAINS.

Typical Colours.
(1) Greys.
(2) Whites.
(3) Yellows.
(4) Light reds.
(5) White with black spots.
Less Common Colours.
(6) Black.
(7) Deep red and red.
(8) Mixed colours, but dark ground or markings predominating.

5. Polled cattle occur in both strains and their crosses. Zebu and cross breds are short or medium horned. The Ankole breed and the crosses that are predominantly Ankole are long horned.

6. Judged by European standards no native cattle in this Protectorate are at present good as milk or beef producers, but some strains are capable of distinct improvement by selection.

7. No reliable tradition or record exists as to the past history of the stock or stock owners of the Protectorate.

8. See reply to paragraph 7. So far as is known no breed or strain ever domiciled in the Protectorate has become extinct.

9. A series of photographs in triplicate illustrating various types of Protectorate stock is attached. These photographs were taken on the Koja Experimental Stock Farm where small herds of the various distinct sub-breeds or types are collected.

10. Normally bulls reach their maximum growth at five years, cows calve down at three years to three years three months as a rough average, though some calve before reaching three years. The Zebu type is generally hardier and has greater powers of resistance to disease and such adverse conditions as poor grazing or fly infestation. In one district, Karamoja, Eastern Province, the cattle (Zebu type) have considerable powers of drought resistance and ability to cover long distances to water.

11. White cattle of the type mentioned in the Questionaire have not been noted.

12. The Ankole cattle as being the largest animals in the Protectorate are probably considered the most valuable, but the question of value depends entirely on local demand and the suitability of introduced stock to live under the conditions where such a demand exists.

13. The best Ankole pastures are principally low ridges of Acacia Savannah country. *Themeda triandra*, the finer Hyparrhenias, and Andropogons, with Brachiarias in shade are the typical grasses. Rainfall occurs in two well defined seasons annually. The average altitude is approximately 4,500 feet.

The Eastern Province pastures, occupied by Zebu stock in greatest concentration, are low lying Combretum Savannahs, taller *Hyparrhenia* spp. and *Panicum maximum* being the typical grasses. The average altitude is approximately 3,500 feet.

14. As already stated the Zebu and the predominantly Zebu strains of stock in the Protectorate are, conditions being equal, more resistant to the majority of stock diseases than the Ankole and the crosses in which it is dominant. East Coast fever, Redwater and Anaplasmosis are enzootic in approximately all parts of the Protectorate except Karamoja district (Eastern Province).

No breed has any marked power of resistance where epizootic trypanosomiasis of the *T. congolense* type is concerned.

15. The Zebu breeds in the Protectorate vary greatly in size, the Karamoja breed being large and the West Nile, Teso, and Sesse Island stock being small.

Of the various cattle-owning communities in the Protectorate only the Bahima of the Western Province and Karamojans can be said to display much intelligence or interest in the management of their stock ".

Two interesting maps appear in the *Annual Report of the Veterinary Department for* 1934. The one shows the cattle population in each district along with information on the racial type of native owner (*e.g.* whether Nilotic, Hamitic, or Bantu), and human populations. In December, 1932, there were 2,151,668 head of cattle in the territory. The other map gives the distribution of the four species of Glossina in Uganda.

Kenya.

Thanks to information supplied to one of the authors (R.W.T.) by the former Director of Agriculture (Holm, A.—File Dairy/3/—), our knowledge of Native Cattle Husbandry in Kenya is considerable. Mr. Holm also kindly supplied 8 photographs, some of which (Figs. 41, 42, 44 and 45) are reproduced here.

Cattle.

As indicated in Map 11, the cattle are of the Shorthorned Zebu type and according to Mr. Holm (letter of 27.8.32) " similar in appearance to the Thar Parkar and Scindi breeds of cattle in India. Although small differentiations appear in different districts, the various types (sub-types) are definitely Zebu ".

As is generally the case, the various sub-types are named after the tribes owning them, thus we have: —(*a*) Boran in the Northern Frontier Province, (*b*) Kavirondo in the South-West of Kenya, (*c*) Nandi also in the South-West, (*d*) Akamba in South-East Kenya and (*e*) Masai in South Kenya.

According to a report of the Veterinary Department dated February, 1932, and entitled *Comparison of Types of Native Cattle at Animal Husbandry Centres*, we learn that six centres have been established under the Veterinary Department " with a view to developing the potential resources of the indigenous cows of the various pastoral tribes for . . . milk production ". Measurements were taken of twenty-five *cows* of certain sub-types and the average is recorded hereunder.

Subtype.	Station.	Height at Shoulder.	Length from Point of Shoulder to Seat of Tail.	Girth.	Breadth of Hips.	Calf at Birth.
Akamba (Fig. 45)....	Machakos........	44″ (111 cm.)	35″ (78 cm.)	54″ (137 cm.)	13·3″ (33 cm.)	—
„	Ngong...........	45″ (114 cm.)	38″ (96 cm.)	58″ (147 cm.)	16″ (40 cm.)	35 lb. (15·8 Kg.)
Boran..............	Isiolo............	45·5″ (115 cm.)	37″ (93 cm.)	56″ (142 cm.)	15·5″ (39 cm.)	38·5 lb. (17·4 Kg.)
„	Ngong...........	50·0″ (127 cm.)	42·9″ (108 cm.)	63″ (161 cm.)	18·4″ (46 cm.)	40·0 lb. (18·1 Kg.)
Kavirondo (Central)..	Maseno..........	44 0″ (111 cm.)	34·5″ (87 cm.)	54″ (137 cm.)	13·7″ (34 cm.)	32·0 lb. (14·5 Kg.)
„ (North)..	Sangalo..........	45″ (114 cm.)	35 5″ (79 cm.)	55″ (139 cm.)	13·7″ (34 cm.)	33·5 lb. (15·2 Kg.)
Nandi (Fig. 42).....	Barraton.........	45·5″ (115 cm.)	36·5″ (92 cm.)	57·7″ (145 cm.)	15·3″ (38 cm.)	41·5 lb. (18·8 Kg.)

Holm (letter Dairy/3/— of 27.8.32) states that colour markings of the various types are extremely mixed and include reds, greys, blacks, black and whites, red and whites, etc. The Boran sub-type is generally putty-grey with a fair proportion of blacks and reds and greys. He adds that good grazing produces a tendency to " go to beef rather than towards extra milk production "(40), and further that " native cattle reach sexual maturity very quickly, although they do not reach maturity as far as growth is concerned until five or six years of age ".

The work referred to above was commenced in 1927, particular attention at Ngong being paid " to impress upon the native mind the extent to which the excessive mortality in livestock could be diminished by the adoption of certain veterinary measures and to demonstrate " the value of controlling breeding and rearing young stock systematically.

In 1928 at the suggestion of Dr. Orr of the Rowett Institute it was decided to educate the native stockowners in animal husbandry especially from the dairy aspect. The position as at February, 1932, was described in a *Report on Improvement on Native Cattle in Kenya Colony*, also kindly furnished by Mr. Alex Holm. Recently, however, Mr. John Anderson (1935) who was taken over from Dr. Orr's staff, has published a valuable paper on *Improvement of Native Cattle by Selective Breeding and Herd Management*, in which he records increased milk yield, by the use of only natural grazing. His summary is as follows:—" The preliminary results here recorded demonstrate that the unselected native cattle(41) bought at random in the reserves, show great promise as producers of milk and dairy products if kept under controlled and regular conditions of management. Under such conditions the milk yield is more than double than that of a native cow under native methods of husbandry. Furthermore, the managed animals become productive at a much earlier age. Though specific age data are not fully available, observation clearly reveals that animals bred from native foundation stock and kept under good management have a much faster rate of growth and attain a much greater body weight. Selected bull calves have been reared which at 16 and 20 months of age weighed 627 and 756 lb.

Although the amount of milk produced is small compared with that produced by imported grade stock, this is compensated for by its very high butter-fat content. Whereas heavy milking breeds usually average a little over 3 per cent. butter-fat, milk from the native cow gives an average of 5·7 per cent. The average yield of butter-fat per cow over all dairy farms in Kenya for 1932 is given as 32 lb. At the Ngong station the average yield for the year 1933-34 was 42 lb. per cow ".

This is surely proof that native cows if submitted to an improved environment will be an asset to the country, whereas if allowed to range without restraint they can only hasten the deterioration of grazing as seen in overstocking and soil erosion. According to Deck (1936) soil erosion and increasing aridity are urgent problems in the Territory, particularly in the Ukamba country where the grazing capacity has already been exceeded to the extent of six times the normal.

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(40) Anderson's work, however, proves that if controlled the cows in an improved environment double their milk yield.

(41) Boran and Masai. In the *Report of* 1932 figures relate to Boran and Machakos (Akamba) heifers.

Much is being done to educate the natives in animal husbandry, the local natives being guided by officials of the Department of Agriculture. Eost Coast Fever, being enzootic in the native reserves, has compelled the authorities to adopt the policy of selection among the native cattle rather than to resort to grading up as in South Africa.

Ghee or clarified butter is the chief native economic dairy product, the output in 1932 from native dairies being estimated at about £25,000. Anderson (1935) estimates that the ratio of milk to ghee is 2·07 gallons to 1 lb. ghee.

As a result of overstocking in the native areas a factory is shortly to be erected to dispose of surplus native cattle in any way that will prove economic, *e.g.* canning, drying of meat, and the manufacture of foods and fertilisers.

Belgian Congo.

Thanks not only to the publications of Carlier (1912) and Le Plae (1933); but also to replies furnished by His Excellency the Governor-General of the Belgian Congo through His Majesty's Consul at Leopoldville (his letter of 19.5.32), interesting information is available regarding native cattle, especially along the eastern frontier of the Belgian Congo(42).

Classification of cattle types in the Congo has been based largely on the absence or presence of horns and in the latter case, whether they are long or short. While the horn length is an important feature, it seems preferable also to consider the general conformation, especially the shape of the skull and the position and structure of the hump. As an example we may mention the cattle of Kivu, which vary not only to the extent of possessing polled as well as horned representatives, but also among the latter in having members with short, medium and long horns. Yet if we group these animals according to conformation they are seen to belong to the Sanga type(43), there being a uniformity in all respects except the horns.

While Epstein believes that the dominant type, the Sanga, arose from the intermixture between the Hamitic Longhorn and the Lateral-horned Zebu (Afrikander) in North-East Africa (probably Abyssinia), Le Plae states that this type " semble provenir de croisements répétés entre des zébus asiatique et des bêtes bovines africaines ". He adds, as we know, that for many centuries there has been intercourse between the people of India and those of East

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(42) We are indebted to Dr. L. Tobback who along with his letter of 26.1.35 sent a useful summary of the position as well as photographs. *Introduction.*

(43) Called Zebu africain (*Bos africanus*) by Le Plae (p. 89).

Africa. It is, however, not clear what were the original "bêtes bovines". For suppose they lacked a hump (as did the Hamitic Longhorn), would they, when crossed with the thoracic-humped Zebu of Asia, produce the cervico-thoracic hump of Sanga cattle? Unfortunately even with the facilities available throughout Africa, but little is known concerning the behaviour of the hump in genetics. It is nevertheless certain that the Shorthorned Zebu of East Africa only differs from its Asiatic relation by containing some amount of African blood, and that owing to its hardy constitution it has replaced the Sanga almost throughout Uganda, Kenya and Tanganyika. Evidently a foothold has also been established in the Belgian Congo, for according to Fig. 40 in Le Plae's work and a description provided by the same and other authorities, the Lugwaret cattle, north-west of Lake Albert, are of the Shorthorned Zebu type.

Other indigenous cattle of Glossina-ridden Congo belong to the Sanga group, those along the mountainous eastern frontier being the most important.

While in Map II the Sanga cattle of Central Africa are referred to as Ankole, this term strictly refers to the Ankole district of Uganda.

Present Sub-types.

Commencing at the northern aspect of the Eastern frontier, and adjacent to the Anglo-Egyptian Sudan, we have the following subtypes:—

1. *Wadai-Dinka.*—Although each of these sub-types is referred to by Le Plae as a "race", it seems desirable to consider them together, for they both occur in the extreme north-eastern corner of the territory, and have several points of resemblance, *e.g.* size, coloration, shape of horns and general appearance. Figs 46 and 47 of Dinka bulls indicate the general type. The influence of the Shorthorned Zebu is seen in the well developed dewlap, but the hump in each case is cervico-thoracic in situation. These cattle are of the same type as found in the adjoining districts of the Sudan.

2. *Lugwaret or Bahu Cattle.*—As mentioned above, north-west of Lake Albert are cattle of the Shorthorned Zebu type and these form an intrusion, in fact, break the continuity of the Sanga line from Abyssinia to the northern end of Lake Tanganyika(⁴⁴). The cattle are small and thick-set (0·95 m. to 1·25 m.) of good conformation, and variously coloured, the most usual pattern being small, black, red or yellow patches on a white ground, very frequently crowded on the flanks. The general appearance is strongly zebu (*i.e.* Shorthorned type) and in males the hump is sometimes very well developed. See Fig. 48 (from Le Plae, p. 98).

3. South of the country occupied by the Lugwaret tribe the cattle are of Sanga type (and known generally as Ankole, Watussi, or Bahema cattle) as far as the north-eastern corner of Lake Tanganyika. According to the reply from the authorities in the Belgian Congo the cattle in the Ituri region are very much mixed as one

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(⁴⁴) Here, due to Glossina, occurs another gap until the Baila cattle (Sanga) of North-East Rhodesia are encountered.

would expect, on the south there being Sanga cattle and to the north Shorthorned Zebu. Chief Blukwa, however, living to the west of Lake Albert, has apparently been so successful in selection that he has built up " une race speciale ". He has selected bulls with short horns and cervico-thoracic humps (in preference to the usual long-horned bull or the Shorthorned Zebu bull), and as will be seen from Fig. 49 (from Fig. 26—Le Plae) the cows are of the same conformation.

As one proceeds south along the frontier, cattle with exceedingly long horns are to be found in the vicinity of Lake Kivu, but they reach their maximum in the herds of Ruanda Urundi (formerly part of German East Africa). Fig. 55 (from Fig. 1, Le Plae) shows two animals of the sacred herd belonging to the King of Ruanda. So striking are these cattle that they have even been featured on the red 60 c. postage stamps of the Belgian Congo. Fig. 54, which is a photograph of the stamp in question, indicates the bull on the right as showing the hump when grazing.

It would appear that the artist has incorrectly represented the position, for it is only the thoracic hump which retains its dorsal position during grazing.

Owing to trypanosomiasis cattle are imported into the Congo from Angola, Ngamiland, and Southern Rhodesia.

The approximate cattle population is 1,300,000, of which a million occur along the eastern frontier.

Tanganyika.

The information available from Tanganyika was supplied by Mr. H. E. Hornby, Director of Veterinary Science and Animal Husbandry in response to the Questionaire. In addition, also to a request from one of us (R. W. T.) through the Superintendent of Education, Tanganyika (Mumford D. B.), for special information on the distribution of white native cattle (see paragraph 11 of Questionaire), a minute from Col. F. J. McCall, former Director of Veterinary Science in Tanganyika, is instructive(45). Both of these are reproduced *in toto*, the former being dated 5th March, 1932, and forwarded by the Chief Secretary, Tanganyika to the Secretary for External Affairs, Pretoria. The latter was dated 12th December, 1930, and forwarded to Pretoria (to R. W. T.) by Mr. Mumford(46).

The cattle population in 1930 was 5,170,162 compared with 1,700,352 about 1913 (*Annual Report for* 1930).

Reply to the Questionaire.

1. If possible, give the origin of recognised types of breeds of cattle.

There are two distinct breeds.

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(45) Mr. Mumford, in forwarding R. W. T.'s request to Col. McCall, mentioned that the distribution of white cattle was important in " view of the light it may throw on movements of tribes ". (Letter Nov. 28th, 1930.)

(46) Interesting observations on Animal Husbandry appear in the *Medizinal Berichte unter die Deutschen Schutzgebiede* of the pre-Great War period.

One is large-horned and small humped and will be referred to as the Ankole breed; the other is short-horned and large-humped and will be referred to as the African Zebu.

The origin of both types is unknown. There are reasons for thinking that the Ankole cattle were introduced only three or four hundred years ago by people known as Bahimas who came from Abyssinia (Le Plae), while the Zebus are undoubtedly closely related to the Indian breeds and probably originated in Asia.

2. Are the breeds or types mentioned under (1) believed to be indigenous or where they imported? If the latter, when and from where?

See reply to (1).

3. State whether the breeds or types mentioned are humped or straight-backed.

The Ankole breed has a small hump approximating to that of the Afrikander. The Zebu has a much larger hump; at its maximum development this is 1 foot high with a tendency to sag.

4. Give a full description of the colour marking of each breed or type mentioned.

The Ankole breed is without distinctive coloration, so that specimens of every ordinary colour common among cattle may be found. Nevertheless whole coloured reds, browns and yellows definitely predominate. There is no predominant colour of the Zebu, and beasts of every colour may be seen; nevertheless local races may have a distinctive coloration, so that Iringa cattle are mostly red; Singida cattle, white; Mkalama cattle, dun; and so on.

5. State whether polled, horned, or a mixture of both, with a description of horns.

Polled animals form a very small minority of the Ankole cattle and are not liked. The horns are in every way an important feature of this breed, and individual animals are known by the shape of the horns.

It is possible to divide the Ankole breed into local races which vary in size of horn: one, the Ishesha, has horns resembling and no larger than the Afrikander's; but other races have enormous horns which, typically, grow outwards, upwards and backwards.

Polled Zebu cattle are rare; the great majority of this breed carry short-horns which grow outwards and upwards, though other shapes such as outwards and downwards are often seen.

6. State whether the animals are of good beef type or milking type; or neither, or both.

Judged by European standards neither breed is of a good beef type, though a fat Zebu ox yields a very fair carcase. Neither breed is of good milking type, and in this respect, also, the Ankole is worse under the usual conditions of seasonal starvation. In good nutritional environment the Ankole is the bigger beast and the better milker.

7. State which of any breed described is supposed to be the original breed and whether such breed or type was founded on other types now extinct; and, if the latter, please supply a description, if possible, of such original extinct breeds or types as can be secured from paintings, rock drawings, and sculpture, etc.

As stated in my reply to question (1), the Ankole breed is believed to be of comparatively recent introduction and so may be called original. The Zebu is almost certainly an offshoot of an Asiatic breed, but whether by intermixture with a type, now extinct, is unknown; there appears to be no archaeological evidence on the point.

8. State which breed or type was first domesticated, the approximate date of domestication, and whether still in use.

See replies to (7) and (1).

9. Please supply photographs in triplicate of typical specimens of both males and females of each breed or type dealt with.

It is regretted that only one photograph illustrating the Ankole breed—an outstanding bull and a good polled cow—can be supplied. On the other hand, a number of photographs of Zebu cattle are appended. These photographs are cut from the 1926 and 1929 *Annual Reports of the Veterinary Department* of this Territory, where they illustrate articles by the former Director, Mr. F. J. McCall, C.B.E., M.C., who was much interested in the subject which forms the basis of this questionaire.

10. Do any of the breeds or types described possess any special features such as slow maturity (say five to seven years) or display extreme hardihood against adverse climatic conditions (such as great heat, drought), disease resistance, ability to range over great distances, etc.?

A characteristic feature of the Ankole breed is its slow maturity under the poor conditions in which it usually lives. Under better conditions this feature is much less marked. The home of these animals is well-watered mountain grassland, and handicapped as they are by great horns they are not suited to withstand hardships. They succumb readily to Rinderpest, and although local races have acquired immunity to tick-borne diseases such as East Coast Fever, this breed should not be considered as disease-resistant, but rather the reverse.

Zebu cattle are on the whole hardier. Local races have been evolved which are highly resistant to many serious diseases and, in addition, maintain their condition when food is scarce and innutritious and when they have access to water only every other day.

11. Special mention must please be made and the fullest possible particulars given of any white cattle with dark points which exist in any of the countries mentioned. By dark points is meant black ears, muzzles, eyerings, or eye lashes, black teats, black hoofs, or spotted or black markings below the knee and hock; also whether any animals answering this description have black skins covered with white hair and whether black spots show through the white hair causing a silvering or spotted appearance, particularly as the animal advances in years.

There are few or no Ankole cattle of this colour, but zebus having white body hairs in spite of a black skin and points are common, particularly in the Singida district of the Central Province. There is nothing radically distinctive about this type of colouring, and in the same herds as animals having it, may be found some with white skin as well as hair (though this is a bad colour, tending to sunburn), and others the visible skin of which is partly black and partly white. Silvering, as seen in the Indian Krishna Valley breed, is rare.

12. Which breed or type described is considered to be the most valuable, and why?

The owners of each breed prefer their own, though one can imagine the owner of a Zebu exchanging it for an Ankole, but not the reverse. From the European point of view the Zebu is definitely the more useful animal; hardier, maturing earlier, milking better, working better and furnishing a better carcase. But it is no use saying this to a native owner of Ankole cattle: he judges altogether differently.

13. Please give the territorial distribution of any type or types described in such a manner as to permit of tracing the areas mentioned on a map. Please also give a description of the areas such as elevation and whether open highland, grass, bush, or forest country; or whether low-lying, hot and sandy, marshy bush or forest country with heavy or low rainfall in each case.

There is a great fly-belt running south from Pasha Gulf of Lake Victoria. The fly-free country to the west of this belt is a strip of mountain grassland along the borders of the Belgian countries of Ruanda and Urundi. This is the home of our Ankole cattle, and it is contiguous with the habitats of the same breed in the Belgian territory and in Uganda. A few herds of cattle of the same breed are found on the eastern side of the Pasha Gulf-Tabora belt, but here they are in contact with and tend to be swamped by the more virile Zebu.

Whereas the Ankole ox is essentially an animal of mountain grassland, the Zebu can adapt itself to almost every kind of vegetation community, and the limiting factors of its distribution in this Territory are neither climatic nor geophysical, but simply tsetse-fly belts.

14. Whether partially resistant to or immune from any disease such as Nagana or other widely distributed and known trouble.

Neither breed has any naturally high resistance to disease, but by selection local races of both breeds have been evolved to overcome most diseases except nagana. Thus all the adult cattle of certain areas are immune to East Coast Fever, but this does not prevent a ninety to one hundred per cent. mortality occurring among other cattle of the same breed moved from a clean to one of these enzootic areas. Odd cattle premunised against a single strain of trypanosome are found near every fly-belt, but herds of cattle immune to all strains of nagana are so rare as to be of negligible importance; the individual animals of these herds are generally stunted by the numerous attacks from which they have suffered and recovered. See, also, reply to question (10).

15. Any other information.

This will be gladly given if asked for and obtainable.

"The Superintendent of Education,
Malangali,
Iringa.

Reference your letter of 28th ultimo.—Native herds of white cattle in East Africa.

The description you give tallies with 95 per cent. of the Boran cattle encountered along the northern border of Kenya. The cattle of Somaliland to a lesser extent fall into the same category. In Tanganyika the cattle of Sekenke, Mkalama and many parts of Singida are in a very high percentage of cases white animals, with dark skins, relatively small horns, have black tips to their tails and ears and very often show black on the extremities of their limbs. Our best herd of native cattle on the Government Stock Farm, Dar es Salaam, namely the white herd, is entirely made up of this type of animal. I have observed that such animals appear to mate most successfully with the Krishna Valley Indian Zebu. Several of this latter breed have been imported by the Government, the cows are usually white but the bulls although as a rule born white with age change through silver grey to dark grey. In the case of the Boran cattle quoted above and also in those of Somaliland, a silver grey coloration amongst oxen is not uncommon, but the characteristic is not so well marked as in the Indian Krishna Valley Zebu cattle. White is a very common colour amongst the cattle of the Wanyaturu of Singida and Mkalama, but further south in Dodoma amongst the Wagogo herds black and whites and brown and whites predominate, whilst in Iringa and the Southern Provinces of Tanganyika, red is the commonest colour encountered.

2. On the whole experience has taught me to expect that in the dry arid sandy countries of low altitude, always provided migrations, wars, disease and other factors have not been unduly operative there, we can expect to find white cattle with dark skins and white sheep with black heads. It would appear that cattle with white coats and dark skins seem able to bear the great heat of these low arid countries better than do other colours. These natural phenomena have been completely obliterated over a great part of the highlands of East Africa probably due to the raids and incursions of the Masai. To-day, according to the Masai, "a good beast can never be a bad colour", with the result that in their herds and in those of tribes such as the Nandi, Algayo, Kikuyu, Warusha, Wachagga, Wakamba and to a lesser extent the Kavirondo, the stock are of most nondescript type.

3. In connection with the cattle of Boran I ought also to have mentioned the cattle of Suk, Tukanna and Karamoja regions. These occupy the hot low country between Mt. Elgon and the Sudan to the North and to the East as far as Lake Rudolph. In this section of the country, whites predominate, but the male stock show signs of darkness and greyness on the back; the horns, however, in these parts are very characteristic, being relatively large (nothing like the size and length of the Belgian Ruanda cattle) with a peculiar uplift which gives the head a hartebeest appearance. Possibly these notes may be of interest, but if Mr. Thornton, Director of Native Agriculture for the Union, wishes further information and cares to write to

me on the matter, I shall be only too pleased to help, for the whole subject interests me greatly. A certain amount of information bearing on native cattle is contained in the various Annual Reports issued by this Department ".

Parish (1917) in discussing the extreme variation of coat colour states: " one other commentary which must be made in connection with colour is that true colours of practically every breed can be seen, Shorthorn reds and whites, Friesland black and whites, Herefords and South Devons, interesting food for thought for those who consider purity of colour is necessarily associated with ancient lineage ".

The two groups of indigenous cattle referred to by Hornby are shown in Figs. 56, 57, 58 and 59, while their distribution is indicated in Map II.

It was in Tanganyika that systematic work was first carried out on native cattle. Soon after the close of the Great War, McCall assembled herds of cattle at Pugu, Dar-es-Salaam and described them with photographs in his *Annual Reports,* especially that for 1926. Unfortunately during the financial depression of 1931-33, these herds were broken up and valuable data lost, but the work has since been reorganised on a smaller scale. Cross-breeding experiments between Shorthorned Zebu and Ankole and between Shorthorned Zebu and European breeds are being conducted and among other things it is hoped that the problem of the hump will be cleared up. Particular attention has been paid to nutrition so that the effects of cross-breeding shall not be affected adversely by a plane of nutrition below the full requirements of the animals. Overstocking with its attendant problems is also receiving consideration (Hornby 1936).

French (1936) has recently published a useful paper on the weights of the various parts of the body of Shorthorned Zebus.

It is significant that after many years experience in Central and East Africa, Hornby (1931) " decided definitely that the occasions when a good purpose is served by issuing grade bulls, derived from crossing indigenous and European breeds, to natives are so few that as a policy such issues are not justified ".

*Angola.**

As indicated in Map II, the cattle of Angola are, according to Dr. Emilio Martins (conversation 6th September, 1934), of two chief sub-types belonging to the Sanga group. These are the Ovambo and Bechuana cattle (see Figs. 64-66 and 67-70).

According to Da Costa (1933) there were 1,500,000 head of cattle in the country.

Northern Rhodesia.

Information on native cattle types was compiled by the Director of Animal Health whose memorandum was despatched by the Chief Secretary, Livingstone, under cover of his letter Vet./A/28 of 18.2.32 to the Secretary for External Affairs, Pretoria. No photographs were available, but a map indicating the Glossina areas and the approximate distribution of cattle was enclosed.

As already described (Curson 1936), the indigenous cattle include the Bechuana sub-type in Barotseland, and the Baila or Mashukulumbwe sub-type between the Zambesi and Kafue rivers. A third group, entirely dissimilar to the two previous, the Shorthorned Zebu or Angoni type occurs " in the Tanganyika plateau and in the Fort Jameson district ". The Bechuana and Baila(47) cattle belong to the Sanga type and possess cervico-thoracic muscular humps (sometimes ill-defined) and large lyre-shaped horns, particularly the former. See Figs. 60-62.

The reply (para. 4) to the Questionaire indicates that the colour of cattle varies greatly as is generally the case among native stock; but it is added that " Chief Lewanika of the Barotse people maintained a herd of pure white cattle with black points. These were all slaughtered when Lewanika died in the year 1916 ".

From a tabulated statement concerning some oxen slaughtered at the Central Research Station, Mazabuka and prepared for the Agricultural Economic Commission of 1931, we learn that two grass fed Baila oxen weighed 872 and 781 lb. The dressed weight was 437 and 440 lb. respectively. At the *same* time grades were slaughtered and " it was hoped . . . that the figures would show that it was more profitable to produce an improved animal ". Too few animals were slaughtered to come to any definite conclusion, but MacDonald, the observer, comments (his letter of 30.6.31 to R. W. T.) that " the animal dressing to the best advantage was . . . the younger Baila (live weight 781 lb.) " The reply to the Questionaire (para. 6) states that the cattle mentioned above " are of the beef type ".

Further details from the reply to the Questionaire are as follows:—

The various types " are slow in maturing. They are extremely hardy and can stand great heat, but cannot remain long without water. All possess a . . . high immunity to tick-borne diseases. The Batonga people who possess Baila cattle do not herd their animals from about the 1st September until the middle of November. During this time they are allowed free range over great distances. . . . The Barotse breed is undoubtedly the most valuable for sale purposes owing to the large size, but unfortunately Pleuropneumonia exists in that Province and the numbers are dwindling year by year. Angoni cattle are not much good for transport purposes, but the Baila breed make exceedingly good trek oxen "!

" All the breeds . . . have a high immunity against tick-borne diseases and it is possible the Baila breed is also partly resistant to trypanosomiasis. In the low-lying districts throughout the territory fluke and worm infestation is prevalent, but no great mortality can be directly attributed to either of these causes ".

412,808 head of the 522,693 cattle are native owned.

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(47) The view is expressed in the reply that the Baila sub-type " would appear to be a cross of both ", *i.e.* the Bechuana and Angoni cattle. Purchase, H. S. (in a conversation on 15.2.35) mentioned that the Barotse cattle are clearly divisible into two groups, the West or Plains cattle with gigantic horns and the East or Forest cattle with small horns.

*Nyasaland.**

Although a Questionaire was not sent to the Nyasaland Administration, it is convenient to include this country in our survey of indigenous cattle.

As will be seen in Map 11 the cattle of Nyasaland are essentially of the Shorthorned Zebu type which has gradually spread from the littoral, and, owing to its hardiness, has displaced the Sanga(48). In fact Shorthorned Zebus are now to be found in North-East Rhodesia as the so-called Angoni cattle.

As the Zambesi river, constituting a formidable barrier to migration especially during the summer months, flows just south of the southern extremity of Nyasaland, it was probably in this vicinity that the Bantu hordes halted and subsequently dispersed in all directions, but chiefly south-east to west in a fanlike fashion. As mentioned previously Sir Harry Johnston believes that this took place about 700 A.D.

Obviously reliable information will never be obtained regarding the details of those prehistoric folk-wanderings, but judging from the present distribution of cattle, the tribes accompanied by the cattle known as Ovambo to-day must have migrated westwards. As described above, the territory they occupied comprises now Angola and South-West Africa Protectorate. Then following the Ovambo herds were the nomads possessing the Bechuana cattle which are to be found to-day in no less than six countries, *viz.*: Angola, Northern and Southern Rhodesia, South-West Africa Protectorate, Bechuanaland Protectorate and Transvaal. Proceeding due south were the owners of what are now the Makalanga and Zulu sub-types, the former keeping to the plateau of Southern Rhodesia while the latter preferred the warmer climate of the coast. It is, of course, also likely that the original owners migrated in the reverse order to that just suggested.

So many factors, *e.g.* distribution of Glossina, stock diseases, wars, further migrations, and climatic conditions are concerned in this problem that it is doubtful whether the veil of obscurity will ever be raised from the events of the past millenium.

At the end of 1934 the cattle in the territory numbered 190,381, of which, 8,597 were European owned (*Annual Rpt. Vet. Dept.* 1934).

South-West Africa Protectorate.

In response to the Questionaire several authorities in the Protectorate expressed their views in regard to indigenous cattle, and the Native Commissioner of Ovamboland (G. Hahn, Esq.) kindly provided photographs. Those who furnished information were Drs. Hans Sigwart (12.11.31), A. Maag (9.11.31) and G. Schmid, Government Veterinary Surgeons, and Messrs. G. Hahn (24.4.32), W. H. A. Schneider (15.12.31) and Carl Schlettwein (29.11.31).

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(48) Mr. S. G. Wilson, M.R.C.V.S., believes that Sanga cattle are also to be found in Nyasaland (conversation).

The data varies in no way from that set down by Groenewald and Curson (1933) in regard to Ovambo cattle, and Curson (1934) concerning Bechuana cattle. The Damara cattle originally brought to the country by the Hereros are of the same type as Bechuana cattle. Both sub-types, the short sturdy Ovambo and the longhorned and longlegged Damara are sub-types of the Sanga group.

It must be emphasised that in compiling the two references just mentioned, valuable assistance was obtained from several of the persons mentioned in the first paragraph.

Photographs of Ovambo cattle appear in Figs. 64-66. The cattle population (1935) is roughly 854,899, including probably 150,000 animals of Ovambo type. Approximately 560,520 head belong to Europeans (letter G4/1 of 3.7.36 from S.V.O., Windhoek).

High Commission Territories.

Nothing has been published by the High Commission Administration regarding the native cattle types of Bechuanaland, Swaziland or Basutoland, nor was a Questionaire sent to any of the officials of the three territories under consideration.

*Bechuanaland Protectorate.**

The cattle of the Bechuanaland Protectorate have been described and the distribution of the sub-type to which they belong has been published in *Farming in South Africa* (Sept., 1934). In other words, the cattle known in Northern Rhodesia as Barotse, those of South-West Africa called Damara, and those known elsewhere as Mangwato or Ngami or Batawana are all members of the Bechuana sub-type which occupies the arid grass region of central South Africa. Figs. 67-70 are sufficient to indicate the conformation.

Of the 1,300,000 cattle (1934) approximately 125,000 were European-owned and of these probably 40,030 were " improved ".

During an official visit to the northern districts of the Protectorate in 1930-31 one of the authors (H. H. C.) not only investigated the Glossina problem, but other factors bearing on the cattle industry; since at the time the export trade to the northern copper fields (Northern Rhodesia and Belgian Congo) was of great economic importance(⁴⁹).

As this aspect has not hitherto been described, it is felt that it may serve as an example of what is required in the way of a description of cattle husbandry.

(⁴⁹) A report dated 19.3.31 was submitted to the Resident Commissioner Bechuanaland Protectorate, Mafeking.

The Cattle Trade in Ngamiland.

Introduction.—The territory generally referred to as Ngamiland corresponds to the Ngamiland (magistracy Maun) and Chobe (magistracy Kasane) Magisterial Districts. These together form the northern portion of Bechuanaland Protectorate and occupy the middle region of the vast Kalahari plateau, an area characterised by the remarkable Okovango Delta.

The extent of the territory is approximately 25,000 square miles and the population numbers probably 25,000 souls, 20,000 belonging to the Ngamiland Magisterial District. The dominant tribe is the Batawana which, however, numbers but 2,000. In fact, the Ngamiland District corresponds more or less to the area known as the Batawana Reserve. Of approximately equal number and of growing importance is the Damara tribe, which was allowed to remain in the territory after the German-Damara campaign of 1904. Since the remaining population, made up of several tribes, is comparatively poor (especially in cattle), we will confine our attention to the Batwana and Damara tribes.

The principal area of occupation in the Chobe District is the right bank of the Chobe River. Here the Batawana, numbering approximately 500, are the principal cattle owners, as indeed they are in Ngamiland proper.

Away from the alluvial banks of the Okovango and Chobe Rivers is flat, waterless, sandy country, covered by dense bush, and occupied by a few wandering Basarwa who naturally possess no cattle (Curson, 1932).

General Remarks.—As far back as the early days of Kimberley, traders were interested in the country. They came with their wagons of goods, bartered them for cattle and returned to the south. Later, stores were established; but it was not until 1920 that the industry received the attention it deserved, this being the year that Messrs. Susman Bros., Livingstone, having lost heavily through Pleuropneumonia in Barotseland, Northern Rhodesia, transferred much of their business to Ngamiland. Others have followed and there is now marked trade rivalry.

According to Stigand (1923), the census of 1921 gave the number of cattle in the Batawana Reserve, which for practical purposes corresponds to the Ngamiland district as 103,989; but he indicates that probably another 50,000 should have been added to this figure. Even to-day it is impossible to give a reliable estimate of the livestock.

For the Chobe district the Acting Assistant Resident Magistrate (Capt. Beeching, R.) furnished the following estimate for 1931:—

Tribe.	*No. of Cattle.*
Batawana	3,375
Basubia	1,323
Bakuba	258
Basarwa (Bushmen)	123
Aliens	307
TOTAL	5,386

The traders naturally prefer barter, but may give half cash and half goods. The Administration, on the other hand, considers cash purchase preferable and in order to stimulate competition has recently granted several new trading licenses. The cash price varies considerably, depending on the centre, the season and the demand, but average figures early in 1931 were as follows:—

> Big oxen, £3. 10s.; other oxen, £2. 10s.-£3. 10s.; Cows £1. 10 s. and Tollies, 10s.-£1.

Care and Management.—With regard to the care and management of cattle, the methods employed by the Batawana and Damara, the chief stock owners, vary somewhat. The *Batawana* being the "aristocracy" of the country, look upon all work as menial, and therefore employ Bakuba or Basarwa herdmen, who, when opportunity offers, would not hesitate to steal a beast, and in any case are neglectful of their masters' interests. The *Damara*, on the other hand, are skilled cattle-men, and through good management their herds have increased from a few hundreds, at the time of their arrival in 1904, to thousands at the present day.

Most of the leading *Batawana* families live at or near Maun, the administrative centre, and their cattle are grazed in the vicinity especially since the Pleuro-pneumonia outbreak of 1927. As a result of overgrazing through concentration of stock, pasture is sought along the edge of the "fly" area where infection by Glossina may take place, particularly along the lower Boro river where conditions for agriculture, too, are excellent. Calves are naturally separated from their mothers and grazed near the homestead. Milking is done as a rule about midday, the native wooden pail being seldom employed (Curson, Thomas and Neitz, 1933). So far has civilisation penetrated that tins or even enamel buckets are used even in the most distant places. After weaning, the yearlings graze with the main herd, but the bulls are not castrated until about 3 years of age, in fact one may see a team of 6-8 bulls ploughing! Under these circumstances heifers calve at an early age, *i.e.* about 2-2½ years. Calving takes place in the crowded bush or palisade kraal, which in the wet season may resemble a morass. Pasture is generally sufficient during the rainy season, but towards the end of the dry season (September, November or even December) it has been exhausted through overstocking. So serious is the position at times, *e.g.* early in November, 1930, there was hardly a blade of grass along the motor road from Tschiri *via* Maun to Lake Ngami (Sihitwa), except along the Gabarachia-Kgoboga section, which had been deserted on account of tsetse-fly. In other words, along a 100-mile stretch, there was no grazing available, 25 miles being occupied by "fly" and the remainder being bare through overgrazing.

Under such conditions not only do hundreds of cattle perish from poverty, their skeletons bleaching along the parched flood-channels, but internal parasites cause a great deal of harm. The natives too, especially the children, suffer much from the loss of milk.

Several Batawana, instead of concentrating their cattle in the southern part of the territory, *e.g.* at Maun or Tsau, send them north to Nokanen and Gomare where losses such as described above are less common. If the cattle posts are frequently visited and the Bakuba and Basarwa servants supervised all will be well, if not, unnecessary losses will take place. A few owners brand their stock in the European manner.

The *Damara*, on the other hand, leave nothing to chance. In the first place, they have not the advantage of grazing and their herds are not welcomed along the fertile swamps. Being strangers they have been compelled to keep their stock in the drier country around Lake Ngami and between this and Tsau. They are, however, more nomadic than the Batawana and will travel many miles to obtain good pasture. The more distant portions and the sandveld, even as far north as 35 miles south of Namaseri, are ranged in order to get grazing. The horizon is eagerly scanned, and if rain has fallen, the Damara mounts his donkey and rides away to investigate what the prospects are. If water has collected in a pan he will bring his cattle and make this his headquarters until he can find better grazing. In the dry season, however, when the sandveld is a barren waste, most rely on the herbage about Lake Ngami, where reeds, rushes, sedges and grass provide sufficient grazing until the wet season. Drinking water is usually a problem and away from the swamp area wells are dug and cattle watered by hand therefrom, a difficult task which the Damara carries out most conscientiously. As would be expected Damara cattle can stand drought conditions better than the Batawana; but in the southern parts of the Kalahari cattle may go for several days without water!

Unlike most South African tribes, the Damara allow, in fact encourage, their women folk to take an interest in their stock. The cattle kraals are large enclosures in which often the family hut is situated, this being done to prevent overcrowding and interfering. It is preferred that cows should calve in the kraal rather than out at pasture, and the milking is done once daily, often at sunrise, by women. The milking pails are of wood and as far as hygiene is concerned, the Batawana is superior to the Damara, for the latter seldom clean the receptacle, in fact it is stated that custom decrees that this should be washed only once annually. Butter is made (by swinging a calabash to and fro) and this is used not only in exchange for grain, but after the addition of some herb, is employed by the women for anointing their bodies. Calves at weaning are grazed with the main herd, any showing trouble in returning to their mothers have a slit cut above the nose and by irritation a knob-like structure eventually forms. Searing or scarifying is often resorted to for conditions such as ophthalmia, lameness, etc., while in several cases cattle are disfigured by the native method of branding, *e.g.* lines along body, cut ears, etc.

Bull calves are castrated at a younger age than under Batawana ownership, from 1-2 years being the usual period. The end of the scrotum is cut off, the testicles are grasped, one in each hand, and with a see-saw-like motion, they are pulled out, the animal in the meantime being firmly secured. A bullock is thrown by seizing the tail, running with it until it stumbles or hesitates, and then swung off its feet when the head is firmly held.

Damaras, not being agriculturists, are generally keen to sell their young oxen, but will seldom part with a cow. As they take to agriculture, there will be less inclination to part with oxen.

Despatch of Export Cattle to Northern Rhodesia.—With regard to trading when the cattle have been disposed of at one of the various trading stations scattered throughout the Delta they are, branded and then inoculated against anthrax. They are next despatched periodically to headquarters, generally at Maun, and after permits have been obtained from the stock inspector, may proceed to their destination, as a rule, north of Kazungula. The journey northwards, however, depends on many factors, *e.g.* state of market, condition of grazing, presence of water, etc.

The firms chiefly engaged in the cattle trade are:—

Messrs. Susman Bros., who are the chief exporters;

Messrs. Orphanides Bros.;

Messrs. R. F. Sutherland, Ltd.; and

Messrs. H. C. Werner, of Lusaka, Northern Rhodesia.

Whereas until about 1920, cattle were exported chiefly to the railway at Palapye Road or *via* Ghanzi and Letututu to the Southern Protectorate, within the past decade the vast majority have been sent north to Kazungula for the Northern Rhodesian and Belgian Congo markets.

Cattle are sent on the long journey of 250-300 miles to Kazungula Crossing (Zambesi River) generally after the rains, as otherwise drinking water would be scanty, in fact there are stretches of country along which, during the dry season, water is not procurable for 2-3 days. In the rainy season, January to April, the greatest distance between any two pools or pans would be 10 miles. In order to encourage the export trade, the Administration has sunk wells at two places, Sakobs and Xana, between Puluhelo and Tsotsoroga Pan, but the whole water problem is exceedingly difficult.

The cattle are sent forward according to size, *e.g.* big oxen, tollies and cows, so as to make grazing easier. *En route* large bush kraals are constructed, not only to prevent animals wandering, but also for protection against lions at night. Each consignment is under the charge of a European foreman who has 2 natives for each 100 head. The cattle move slowly along at the rate of 10-15 miles *per diem*, unless circumstances ordain otherwise; but obviously the condition of the stock must not suffer in any way. When the market is slack, as it has been since 1930, the cattle are grazed in suitable localities, *e.g.* along the Chobe river, between Kachikau and Kazungula, until they are required. The route taken by cattle is along the so-called cattle or "fly"-free road, and shown on the accompanying map, Fig. 72. It will be observed that as far as Makarane, the motor and cattle routes are widely separated, except at Tsotsoroga Pan, but north of this, they cross frequently. Owing to the sandy nature of the country, cattle are kept from the motor road otherwise it would be rendered useless for motor traffic. A dipping tank of 5,000 gallons is available at Kasane, 9 miles from Kazungula, but parasitic conditions, *e.g.* due to ticks, lice and acari, are chiefly seen in winter.

All slaughter cattle proceeding to Northern Rhodesia or the Belgian Congo, are quarantined a month at the Northern Rhodesian Quarantine Station at Kazungula. On the Bechuanaland Protectorate side of the Zambesi river, a Government Veterinary Officer is stationed at Kazungula (just opposite the Rhodesian Station), and he is responsible for the health of the animals prior to the crossing which is carried out towards the end of each month. Before the cattle are swum across, they are inspected by a veterinarian of the Northern Rhodesian Government and then branded with the letter K, on the right hindquarter, the latter operation being carried out in a crush by a stock inspector of the Bechuanaland Protectorate Government.

The crossing of the Zambesi river, which at this point is approximately 600 yards wide, is carried out by natives who, standing in a large barge, attach ropes about the horns of the cattle. Other natives then paddle the barge across stream, the animals really being pulled along. Five to seven head are taken at a time and the journey across occupies 15-20 minutes, whereas the return trip is done in half that time. On reaching the Northern Rhodesian side, a shelving bank is selected and as soon as the cattle feel the ground, the head ropes are loosened (one boy to each beast), and they walk ashore without difficulty. The Quarantine Station, in charge of a stock inspector, is situated about 50 miles from Livingstone and has a river frontage of about 6 miles. Dipping is carried out weekly, and before cattle are released inspection by a veterinarian is again carried out.

The fees for the crossing are as follows: 1s. per head to the Bechuanaland Protectorate Government—supervision or inspection fee, 1s. per head to Messrs. R. F. Sutherland & Co., who carry out the crossing, and 3s. per head to the Northern Rhodesian Government for inspection, grazing and dipping. The Bechuanaland Protectorate Government fee is paid to the Magistrate at Kasane, and herd boys during the quarantine period of one month are provided by the owners.

The cattle on arrival at Livingstone are either killed for local consumption (since Northern Rhodesia can hardly supply its own needs), or sent in sealed railway trucks to the copper mines in Northern Rhodesia or Belgian Congo. Each train is under the charge of a policeman who has instructions to shoot any cattle which may escape from a truck, *e.g.* as the result of a derailment. It has been suggested that breeding stock from Ngamiland should be exported to the north. Not only is this undesirable at present, for the reason that it is not certain whether Ngamiland is in a position to do so, but since the Northern Rhodesian authorities suggest a six months' quarantine, it is perhaps better to postpone this plan until the matter has been further considered.

Available export totals *via* Kazangula are as follows:—

1920	1,931 head.	1928	5,882 head.
1921	3,900 ,,	1929	4,405 ,,
1922	3,629 ,,	1930	7,386 ,,
1923	6,302 ,,	1931	9,090 ,,
1924	6,669 ,,	1932	6,392 ,,
1925	7,541 ,,	1933	—
1926	9,914 ,,	1934	1,800 ,,
1927	6,510 ,,	1935	No record.

Ngamiland, being so isolated and having no centres of European activity, *e.g.* mining or factories, depends entirely on the cattle industry. If there is no market for their stock, the natives are unable to pay their annual tax of 25s. The traders, having invested large sums of money in the country, have made serious efforts to improve the position by finding new markets. Attempts have even been made to establish business relations with the diamond mines in Angola, the cattle being driven through the Caprivi Strip *via* Bagane Drift, and thence along the right bank of the Kwando River to Saurimo, a distance of over 1,000 miles.

In the circumstances, the interest of the Bechuanaland Protectorate Government in the cattle trade can well be understood.

Vicissitudes of Cattle Husbandry.—Since the eradication of Pleuro-pneumonia (*intermane*) at the Caprivi border in 1927, there is probably no healthier cattle country in Africa. The cause of the greatest mortality is starvation (*libupamau*), but this is due to the concentration of herds in the south of the territory, leaving untouched the rich pastures north of Gomare. Following on the weakened state associated with malnutrition, many animals in seeking water are unable to extricate themselves from the muddy " melapo " and thus die of exhaustion. Nagana (*tsetse*), *i.e.* chiefly *T. congolense* disease, is responsible for comparatively few deaths especially now that the value of trypanocides is widely known. In any case, by careful herding Glossina infection may be avoided. Anthrax (*kwatshi*) is reported to be prevalent at Kachikau, Maun, Toten, Lake Ngami and Tsau, but it is probably less serious than is believed. Anthrax vaccine is issued free to natives and all export cattle are inoculated at Maun. Mange and lousiness are common in winter. Export cattle while in the vicinity of Kasane (where the only cattle dip-tank is situated) improve after a few immersions. Fascioliasis and other worm infections are common in the swamp region. " Bad " water (whether due to mineral, animal or vegetable matter) is undoubtedly responsible for mortality. It is more than likely in some cases that decomposing algae are harmful and that after drinking from polluted ponds death follows after a short illness. Pammel (1911) records *Nodularia spumigera* as being suspect in Australia, and there is reason for believing that a similar agent has been encountered in South Africa (*e.g.* Worcester). Poisonous plants also play an importat rôle. The chief malady affecting young cattle is calf paratyphoid (*sibiti*).

*Basutoland.**

Owing to Basutoland being surrounded by Union territory, and cattle of European breeds having been introduced in large numbers over many years, the original native cattle, formerly chiefly of Zulu conformation, scarcely exist. In their place are the usual nondescript animals tending towards the Brachyceros type.

*Swaziland.**

As will be seen from Map II, the cattle of Swaziland are of the Zulu sub-type.

Southern Rhodesia.

The reply (letter of 5.11.31) from the Prime Minister, Southern Rhodesia, to the Minister of External Affairs, Pretoria was to the effect that the native cattle of Southern Rhodesia had already been described by Nobbs (1927) and that the Government was not in possession of a copy of the paper in question. The reference, however, was given.

Unfortunately Nobbs' original article was not accompanied by photographs nor by a map showing distribution of cattle types, but in subsequent papers by Bisschop and Curson (1933) and Curson (1934) this information has been provided.

All that it is necessary to state now is that there are two distinct types of native cattle, the Bechuana beast and the so-called Makalanga or Mashona beast.

Nobbs (1927) described the Bechuana as the Mangwato or Amabowe and his comments are as follows:—" The Mangwato is a distinct type, deserving of the distinction of a breed, not to be confused with the Matabele(50) or Makalanga. It is found (in Southern Rhodesia) particularly in the south and west of Gwanda district, in the southern end of Bulalima-Mangwe, and across the border to the south of Tuli also. This breed is one which has contributed its share to the Matabele, and was in existence long before the advent of that warrior race. Mangwato cattle are large-framed, the fat oxen averaging 600 lb. dressed weight. They stand on long, strong limbs and carry very wide-spreading thick, heavy horns. They make heavy, powerful trek oxen of great endurance, and the carcase is of good shape and quality, though maturity is only reached at eight or nine years. The cows yield, as native breeds go, a good quantity of rich creamy milk, and generally furnish about ten calves. They show a resistance to the common diseases, and probably possess some degree of inherited immunity. Red or red-white colours predominate though most colours are found, and they show that fixity of type which is characteristic of long-established breeds ".

(50) Nobbs describes the Matabele cattle, but as he himself notes, " they are of a very mixed derivation so recently collected and amalgamated that the original types are known and distinguished, and they possess no common characteristics distinguishing them as a breed . . . "

He makes no reference to the presence of a small hump, cervico-thoracic in situation and muscular in structure, an important feature of Sanga cattle in general.

The Makalanga cow was described by Bisschop and Curson (1933) and it remains to be stated that the bull, like the cow, varies greatly in conformation. All Makalanga cattle are small and of light build, a good ox rarely exceeding 800 lb. Some animals are sturdy while the majority are weedy. The cow shown in Fig. 74 is a good example, but the bull represented in Fig. 73 has poorly developed limbs. The distribution of the type is shown in Map II.

In regard to *origin* of cattle in Southern Rhodesia we may quote Hall and Neal's (1902) ([51]) remarks concerning the ruins of that country as follows:—" In the more ancient débris heaps and under ancient cemented floors are *horns* of very small oxen—short horned—smaller than Guernsey cattle and probably the breed from which the present Zambesi cattle originated. They were preserved by the cement-work by which they were hermetically sealed from the action of the weather. Long-horned cattle were not introduced into South Africa until late in the seventeenth century " (p. 153).

Until the horns have been examined it is difficult to pass an opinion, but assuming they belonged to cattle is it certain they were not of calves, which according to our view were of the Sanga type? It has already been stated above that long-horned cattle were introduced south of the Zambesi first by the Hottentots and later by the Bantu. It was only in the seventeenth century that Europeans introduced cattle into South Africa.

In connection with prehistoric ruins one should read Gwatkin's (1933) articles in *The Rhodesian Mining Journal* on " The Ancient Forts of Penhalonga " in which he discusses the view that they were cattle pits. Another paper by Capt. Wilson entitled " The Ancient Civilisation of the Rift Valley " and appearing in *Man* (Vol. 32, p. 298) suggests a prehistoric Great North Road to Egypt. Apart from Egypt where valuable historical records are represented on ancient monuments, nothing is known elsewhere in Africa regarding the earliest cattle, except what is indicated in this Report.

Of the 2,716,762 cattle in the territory in 1934, 1,708,607 were native owned.

*Mozambique.**

Map II does not represent at all accurately the distribution of cattle in this territory. All that can be stated is that the Zambesi River forms a convenient line of demarcation between the Short-horned Zebu to the north and the Sanga cattle (sub-type Zulu) to the south. According to Botelho (1933) there were approximately half a million head of cattle in the country in 1932, distributed as follows:—

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[51] This reference was brought to our notice by a correspondent signing himself " L. F.", his letter being dated 29.3.33.

Lourenco Marques	375,770
Inhambane	15,944
Quelimane	12,076
Tete	72,462
Mozambique	4,817
Cabo Delgado	—
Niasa	2,013
Manica and Sofala	36,067
TOTAL	519,149

*Union of South Africa.**

As described by Curson (1936) "the time has long passed for describing the indigenous cattle of the Cape, Orange Free State and Southern Transvaal. Apart from the scattered Afrikander herds, there has been so much intermingling with cattle of European origin that a nondescript type, possessing chiefly Brachyceros characteristics, has arisen "(52). As shown in Map II the cattle of the Northern Transvaal are of Bechuana origin, and those of Natal of Zulu type.

As photographs have already been given of Bechuana cattle (Figs. 67-70), it is necessary only to show the Afrikander (Figs. 77 and 78) and Zulu (Figs. 79-82). The Zulu beast is a member of the Sanga type and as mentioned in the classification (p. 11), while the hump is cervico-thoracic and muscular, the skull is quite different, the circular horns, especially in the cow, being upright and lyre-shaped. The Afrikander on the other hand has oval horns which are disposed in a lateral direction. Again the convex profile of the elongated Afrikander skull differs materially from the straight profile of the broad Zulu skull.

Figures 81 and 82 are photographs of a bull and a cow of the Black "Nkone" breed which by selection has been evolved from the Zulu sub-type. A herd has been establised at Onderstepoort.

Thompson (1932) has described a herd of so-called Sacred cattle of Sanga type in the Transkeian Territories. These Bolowana cattle as well as another herd described by Soga (1931) apparently represent the most southerly of our indigenous cattle, in fact, they may be compared to islands in a sea of nondescript grades.

SUMMARY.

It being believed that the Third and Fourth Migrations were responsible for the extension of indigenous cattle from North-East Africa (probably Abyssinia), the cattle types occurring in the various territories concerned may now be summarised:

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(52) Centres of such nondescript cattle are to be seen throughout Africa in the neighbourhood of towns. The ubiquitous European, wishing to improve the milk yield of native cattle, soon introduces Brachyceros breeds.

Third Migration.

It is remarkable that the Lateral-horned Zebu or Afrikander is found only in the Union of South Africa, chiefly in the north-western part of the Orange Free State.

Fourth Migration.

Sanga cattle occur in Central and South Africa either alone or associated with the Shorthorned Zebu, which came apparently from India and constituted the Sixth Migration.

Abyssinia.—(1) The cattle are of three types, Shorthorned Zebu, Brachyceros and Sanga, the last-mentioned receiving its name from the giant horned Galla or Sanga oxen of Abyssinia.

Anglo-Egyptian Sudan.—(1) The same three types occur in the Sudan.

Uganda.—(1) The Shorthorned Zebu is dominant in the east while the Sanga (Ankole) is most numerous in the south-west.

(2) Crosses are numerous. These naturally tend either towards the Sanga or the Shorthorned Zebu side.

(3) At the Koja Experimental Farm observations are made into native cattle.

Kenya.—(1) The cattle of Kenya are all Shorthorned Zebus.

(2) Much experimental work is being undertaken in order to improve the milk yield of native cattle, the results being most promising.

Belgian Congo.—(1) Sanga cattle, which vary from polled animals (a few) to giant horned specimens in Ruanda-Urundi, occur along the mountainous eastern border.

(2) Apart from the eastern frontier and a few small centres elsewhere, *e.g.* Katanga, the country is Glossina-ridden.

Tanganyika.—(1) The dominant type is the Shorthorned Zebu, only a small part of the territory in the north-west being occupied by Sanga (Ankole) cattle.

(2) More attention has been paid in Tanganyika to native cattle problems than elsewhere in Africa.

Angola.—(1) The cattle are entirely of Sanga type, the sub-types represented being the sturdy Ovambo and the long legged Bechuana.

Northern Rhodesia.—(1) Both Shorthorned Zebus and Sanga cattle occur, the former in the north-east being an extension of the Sixth Migration, and the latter resulting from the Fourth Migration.

(2) Two sub-types of Sanga are to be found, the Bechuana or Barotse in the west and the Baila (or Mashukulumbwe) in the central region.

Nyasaland.—(1) The dominant type is Shorthorned Zebu.

South-West Africa Protectorate.—As in Angola, two sub-types of Sanga cattle are to be found, the Ovambo in the north and Bechuana (or Damara or Herero) in the central areas. In the south the cattle are nondescript as in the Cape Province.

High Commission Territories, Bechuanaland Protectorate.—(1) The dominant sub-type is Bechuana which offers much promise of improvement by selection.

(2) A description is given of the cattle export trade of Ngamiland.

Basutoland.—(1) The cattle are nondescript as a result of influence of European breeds.

Swaziland.—(1) Sanga cattle (Zulu sub-type) predominate.

Southern Rhodesia.—(1) Two sub-types of the Sanga occur, namely, the Makalanga (or Mashona) and the Bechuana.

Mozambique. (1) The cattle are of Sanga type south of the Zambesi River (Zulu sub-type), but of Shorthorned Zebu type north of the river.

Union of South Africa.—(1) The greater part of the country is occupied by cattle of nondescript type, originally Sanga, but gradually tending towards Brachyceros.

(2) The Afrikander is the native type which through selection by European farmers has attained universal renown. The second indigenous variety hitherto neglected is the Zulu, a sub-type of the Sanga.

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(C) FIFTH AND SIXTH MIGRATIONS.

All that is necessary to state is that, judging from the present distribution of cattle, when the Bantu people migrated from North-East Africa, not only did they proceed southwards (as described under the Fourth Migration) but also in a westerly direction. Evidence for this is provided by the fact that the Bornu cattle of Northern Nigeria or Kouri cattle of French Equatorial Africa are of Sanga type and must have come from probably Abyssinia. This constitutes the so-called Fifth Migration. The Sixth Migration refers to the westerly passage of the Shorthorned Zebu along with Arab owners from the coast of East Africa, probably from the North of Kenya. If this route were followed towards Lake Chad the Glossina regions of the Southern Anglo-Egyptian Sudan and French Equatorial Africa would have been avoided, *i.e.* assuming that the northern limits of tsetse-fly have not altered much within the past millenium.

The lyre-horned Zebu appears to represent the cross between the Hamitic Longhorn and Shorthorned Zebu.

Summary.

As we have seen there are cattle of Sanga and Shorthorned Zebu types in West Africa. Knowing that these are best represented in North-East and East Africa, it is suggested that they accompanied the wanderings of Bantu tribes and Arab warriors from North-East and East Africa respectively.

CHAPTER III.

SOME EXTRA-AFRICAN CATTLE.

INTRODUCTION.

As it was obvious that certain breeds of cattle outside Africa bore a striking resemblance to some African types or sub-types, it was decided to seek information from the countries in question so as to acquire knowledge as to their origin. In this way it was hoped light might be thrown on the origin of African cattle.

At this point it should be stressed that Curson and Epstein's paper (1934) on the skull of the three parent stocks of African cattle with comments on migration routes, and Curson's paper (1935) on the hump of African cattle, had not appeared, so the position regarding origin was not so clear then as it is to-day.

Accordingly a Questionaire was sent to the countries under consideration. Thanks to the Secretary of the Imperial Council of Agricultural Research, New Delhi, replies were obtained from the Directors of Agriculture of Madras, United Provinces and Bombay. As is well known to those who have been to East Africa, the native cattle are of the so-called Indian Zebu or Brahman type. Hence our desire to approach the Indian authorities. Again, owing to white cattle (Fulani) with pigmented skins having been encountered, in Nigeria (during a tour of that country by R. W. T. in 1911) and Zululand, and being aware that cattle of similar coloration occurred in Scandinavia, a Questionaire was despatched to both the Swedish and Norwegian Boards of Agriculture. Finally, having observed in the Argentine (during a visit to that country by R. W. T. in 1927) that "Criollo" cattle possessed skulls similar to those frequently seen in African Sanga cattle, it was decided to approach the Minister of Agriculture, not only in Buenos Aires but also at Rio de Janeiro, since "Criollo" cattle are to be found in Brazil as well.

So generous was the response to our enquiries that it will be impossible in an account of this nature to discuss anything but that which bears on African cattle. Not only were complete accounts furnished in most cases of the breeds of the various countries but excellent photographs accompanied the descriptions. To the authorities of the respective countries we extend our cordial thanks.

It will be convenient, however, to refer to data available from Great Britain, Palestine and Jamaica although no Questionaire was sent to those countries.

India.

It is not generally known that, of the 186 millions of cattle in Asia, India possesses the largest number, namely 167 millions. In fact India is the greatest cattle country in the world, the United States of America coming next with 61 millions (*Vet. Rec.* 18th April, 1936, p. 517).

As mentioned on p. 623 the Indian Zebu or rather the Shorthorned Zebu is characterised by an elongated skull with generally a convex profile, and a musculo-fatty thoracic hump. On dissection, the thoracic vertebrae are seen to possess bifid superior spines, as in the Afrikander. It must be emphasised that not all Indian cattle are shorthorned. Some breeds, *e.g.* Hallikar and Amrit Mahal have long horns, while according to Epstein (letter of 10.4.36 to Director of Veterinary Services) in a Nepalese breed the skull resembles that of the Afrikander. See *Onderstepoort Journal*, Vol. 7, No. 1 (1936).

After reviewing the particulars forwarded from India it was obvious, as stated by Holm (see Kenya), that the Indian breed resembling most closely the cattle of East Africa was the Thar Parkar. See Fig. 83. A description of the breed, taken from leaflet No. 19 of 1926 and issued by the Department of Agriculture, Bombay, is given hereunder:—

" *Breeds of Cattle in the Bombay Presidency—*
8. *The Thar Parkar Breed.*

The Bombay Presidency to-day possesses some eight breeds of cattle, which undoubtedly at one time must have been pure breeds, as to-day in certain localities fairly pure types of these breeds are to be found.

Sporadic reappearance of typical animals is to be found in each breed, undoubtedly reversion to the one-time pure breed. These, however, are getting few and far between owing to in-breeding in the mongrel herds to be found to-day.

These breeds are or have been so bred that they are particularly useful to the tracts in which they are found, and it is very doubtful if any other breed could give the necessary good work that the breed now found in the locality is capable of. To illustrate, the long legged, upstanding Kankrej is particularly suited to the deep rutted sandy roads of Gujerat, and the Dangi with its flint-like hoof, and its dark skin is particularly useful in the heavy rain Ghat tracts and wet rice cultivation of these areas.

The breeds of the Presidency can be divided into the following grades according to their usefulness:—

Heavy, Light and Medium *draught, milking* or *dual* purpose:

The Heavy *draught* breeds being the Kankrej, Krishna Valley and Gir Breeds.

The Light fast *draught* breeds being the Amrit Mahal and the Khillari.

The Medium *draught* breeds being the Dangi.

Purely *milk* breeds being the Sindhi.

Dual purpose breeds being the Gir, Kankrej and Thar Parkar.

Introduction. The breed takes its name from the District Thar Parkar in which it is bred. From the outward conformation of the animal and from the history of the breeders, it is fairly certain that this breed has sprung from the Kankrej. The Sindhi breeders of Badin some generations ago settled in Radhanpur State and then later migrated to Badin, and it is more than possible they took with them this fine breed of Kankrej cattle. The Mahomedan breeder has no scruples as to milking cattle, milk being his first object in keeping cattle. Unlike the Kankrej of Gujerat, the Thar Parkar is a first class milk animal. The Thar Parkar was brought into prominence during the Great War when milch cattle were required for the army in Mesopotamia. On an average it did better in Mesopotamia than the famous Sindhi and has proved itself at many of the military farms as a good dairy animal.

Natural Habitat.—The home of the Thar Parkar is in the Thar Parkar District of South Sind, where only scanty and rough grasses grow which is so peculiar to this district. The soil responds quickly to even light showers of rain. The district is extremely hot in the summer and fairly cold in the winter, the nights being cool throughout the year. The tract being very difficult to get at and transport facilities being very poor, there has been little chance of any foreign breed getting into the tract and it has, therefore, kept fairly pure.

Breeders.—The breeding of this breed is in the hands of a professional Mahomedan breeder, who, like the breeder of the Sindhi breed, locates his temporary home, where water is available. He is particular about the sire that heads his herd.

Types.—There are two types of this breed, one inclined to put on more flesh than the other, in colour and outward conformation there being little or no difference. The types to be had at Mithi, Dipla and Nagar Parkar where the water is brackish are not so good as those bred in other parts and these are inclined to be more angular than those bred in other areas.

Availability.—The breed, although bred extensively, seldom finds its way far from its natural home, this being due to the nature of the country. Purchases can be made at the following villages which are railway stations:—

Badin, Dhoro Naro, Jida Gudam and Shadipali.

An annual fair is held at Badin in November, where a good number of breeders assemble with their cattle. A small herd is maintained at Phihai, where bulls will be available in the near future. The Imperial Department of Agriculture have a fine herd in Karnal in the Punjab.

Prices.—The prices of the male range between Rs. 200 to 250, the cow about Rs. 150 to 200, and a good pair of bullocks being valued at about Rs. 300 to 400.

As a Draught and Milch Animal.—The size, hardiness and general conformation and the milking qualities make this breed one of the most useful in India. It is now being recommended by the Imperial Department as the breed to be used to improve all other breeds in the country. It has proved its usefulness as an economical dairy animal in Mesopotamia and all over India. At one time it was known as a grey Sindhi and was confounded with the Sindhi, until the Great War when they did so well, the matter was investigated and it was found that it was a breed, bred in a special locality. In Karachi it is often spoken of as the " Cutchi " and is used extensively for road draught. The great difficulty is in purchasing good specimens as the greater portion of breeding is carried on in the interior miles away from the railway.

The Thar Parkar as a dual purpose animal is second to none in India.

Description.- A fair sized animal with good care will grow to a large size, the cow weighing from 850 lb. to 950 lb. and bull from 950 lb. to 1,200 lb.

Colour.—Typically a grey with dark or black points, the fore-quarters and hind-quarters being almost black. The colour in the female like all greys gets deeper in the winter and in-calf, after calving getting lighter in colour.

Head.—The head is well carried, small with a distinct dish in the centre, medium muzzle, nostrils broad, eyes small but bright, the horns are irregular, large and small horns being found, ears medium to large and pendulous.

Fore-quarters.—Neck: fine, well set on, with thin fine medium dewlap. Fore-quarters, square, strong and withers light, hump of medium size.

Hind-quarters.—Fairly wide, long, fairly level, rump long and muscular in the male; fine, broad in the female.

Udder.—Wide, long, tucked up, small, but very shapely with good teats well set apart, milk veins prominent and tortuous.

Hair and Skin.—Hair very fine and the skin is thin, fine and pliable.

Style. Alert, vigorous and bold.

As has been mentioned, a fine dual purpose animal suitable to any part of India. It has the makings of a first class dairy animal and the males really good draught animals ".

Although descriptions of other breeds of Shorthorn Zebu cattle, *e.g.* along the Arabian coast, are not available, yet considering the extent of trade between the East Coast of Africa and Bombay over many centuries it would not be surprising that cattle of the Thar Parkar were originally introduced into what are to-day Kenya and Tanganyika. What is indeed surprising is that the African cattle have after so many centuries retained in general their original conformation! The influence, however, of inter-breeding with Sanga cattle is manifest in many districts.

It is worth noting that McCall (1930) imported Krishna Valley bulls from India in order to improve the Tanganyika Zebu, but according to Hornby (1933) " an unexpected setback . . . is the discovery that the grade offspring are extraordinarily intractable, and we are now inclined to think that the greater docility of the average Tanganyika Zebu is a very valuable attribute which outweighs any small advantage which the Krishna Valley may have in conformation or milk supply " (p. 72).

White cattle with pigmented skins occur not only in India, but also in Tanganyika, north of the Central Railway (Singida cattle), and Nigeria (White Fulani cattle). While in these cases the African cattle are of Zebu type (thoracic hump) the question of their origin from India and their relationship is possible. The White Zulu cattle (Nyoniaipumuli cattle) on the other hand are of a different type (as is the Fjällras of Sweden), and in this case it is not a question of the Zulu cattle originating in the East and of relationship, but of the *origin of the type of coloration*, namely, white coat with pigmented skin. More will be said about this matter in the reference to Sweden.

*Palestine**(53).

The undermentioned breeds of cattle are found in Palestine:—

(*a*) Small local cow—Arab breed.
(*b*) Jaulan breed.
(*c*) Beyrouth or Lebanese breed.
(*d*) Damascus breed.

(*a*) *Arab Breed.*

General.—This breed is neither a dairy, beef or working animal, due to poor feeding and housing conditions and to no attempt at selection or use of mature bulls. This breed has one good quality, however, in that it is exceptionally hardy and is very resistant to disease.

Conformation:	Height at withers	108 cm.
	Height at back	107 ,,
	Height at croup	111 ,,
	Breadth of chest	30 ,,
	Depth of chest	56 ,,

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(53) Details kindly furnished by J. M. Smith, Esq., O.B.E., C.V.O., Palestine (his letter V/8/2/20 of 8.3.35).

Colour.—The usual colour is black and white but red cattle with white markings are also found.

Weight.—Average live weight is 200-250 Kg.

Milking Qualities.—The annual milk yield ranges between 400 and 800 litres with 4 to 5 per cent. butter-fat.

(*b*) *Jaulan Breed.*

General.—This breed originated from Jaulan (Syria) and is superior to the Arab, being of a more robust build, the cows giving more milk and the oxen better for draught work. Like the Arab, the breed is also resistant to disease.

Conformation :—

Height of withers	114	cm.
Height of back	114	,,
Height of croup	115	,,
Height of tail-head	117	,,
Breadth of chest	32	,,
Depth of chest	57	,,
Circumference of ribs	155	,,

Colour.—Red and fawn is usual but black is not exceptional.

Weight.—Live weight varies between 350 to 450 Kg.

Milking Qualities.—Under their home conditions cows yield from 700 to 1,200 litres.

(*c*) *Beyrouth Breed.*

General.—This breed originated in the Lebanon and were bred mostly by small holders in the villages of the Lebanon, especially around Beyrouth. This breed is larger than the Jaulan and has been imported into Palestine because of its milking, draught and disease-resisting qualities.

Conformation :—

Height of withers	125	cm.
Height of back	124	,,
Height of croup	127	,,
Height of tail head	129	,,
Breadth of chest	88	,,
Depth of chest	65	,,
Circumference of ribs	174	,,

Colour.—Red and fawn is usual but black is not exceptional.

Weight.—Live weight varies between 350 to 450 Kg.

Milking Qualities.—Under usual pasture conditions the yield is between 1,000 to 1,500 litres. When hand-fed with concentrated food the yield goes up as high as 4,000 litres.

(*d*) *Damascus Breed.*

General.—This breed originated in Syria, principally around Damascus, and was imported into Palestine because of its high milking qualities. The breed is inclined to be leggy, not very solidly built and is not so resistant to disease as the other breeds. It is primarily a milk breed and is not of much value for beef and draught purposes .

Conformation:—	Height of withers	140 cm.
	Height of back	139 ,,
	Height of croup	144 ,,
	Breadth of chest	37 ,,
	Depth of chest	69 ,,
	Circumference of ribs	179 ,,

Colour.—Black or red.

Weight.—The average live weight is 470 Kg.

Milking Qualities.—The average yield is 3,000 litres but selected cows give as high as 5,000 litres.

Thanks to the photographs sent by the Chief Veterinary Officer, it is clear that the cattle of Palestine are of Brachyceros stock.

Norway.

Of chief interest in Norway and Sweden are the white cattle which one of us (R. W. T.) believed might be related to the white cattle of Africa. Subsequent investigation, however, indicates that the cattle are of entirely different types, but this does not rule out the possibility of the white colour and pigmented skin having in each case been derived from the same source, presumably in Asia.

Professor H. Isaachsen of the Royal Agricultural College, Aas, Norway, not only kindly replied (27th February, 1932) to our Questionaire, but also furnished two publications "Landbruksboken" and "Husdybruket" describing the Norwegian breeds, of which there are six, each occupying a well defined part of the country.

He states that there is "no breed of the type generally called white . . . we have—as in Northern Sweden, Northern and Eastern Finland and Northern Russia—what we call 'sidet' (sided) colour type *i.e.* the sides are black, red or grey. The back has a broad white line from the head (generally the line begins a little back of the neck) down to the tassel of the tail. The under part of the body from the head or neck is white. These coloured sides are of various sizes until there are only small patches which finally in some cattle are absent; and the animals are then all white, but with pigmented ears, and generally pigmented muzzle. The white cattle in this country are accordingly white, because they are decoloured from the 'sidet' type, or to put the matter in another way, the white cattle have only white markings! As a type our cattle have no eye rings or black teats, but there might be some animals possessing these dark points.

We have no animals with black skins covered with white hair. Sometimes, however, about one inch outside the coloured sides of the 'sidet' type, the skin under the white coat is pigmented. Ears, hoofs, etc., are pigmented, dark or less dark according to the main colour of the animal. . . . The muzzle is either pigmented or flesh coloured".

In Norway no attempt has been made to create a breed from the white cattle.

Sweden.

The Swedish authorities kindly replied to the Questionaire and provided copies of the publications " Svenska husdjurraser " by Sven Sixtensson and " Agriculture and Fisheries in Sweden ", published by the Swedish Board of Agriculture.

In Sweden, according to Professor Isaachsen, the Fjällras or Fjreldras polled mountain breed (see Figs. 84 and 85) represents the " sidet " type just referred to. The coloured parts may be black, red or grey, but apparently through selection chiefly white animals with black points are preferred. There are two other breeds, namely Swedish red and white breed and the Swedish lowland or Fries breed.

*Great Britain.**

We were naturally interested in the so-called wild white cattle of Great Britain for the advancement of which in 1918 was formed the Park Cattle Society. Much useful information was obtained through the Office of the High Commissioner, Trafalgar Square, London, from Sir Claud Alexander, who has probably the largest herd in the United Kingdom.

Bohrmann (1924) has contributed an article on Park Cattle, but it suffices to state at present that there are two varieties, polled and horned, and that only the points, *e.g.* muzzle, feet and ears are pigmented—not the entire body as sometimes seen in African white cattle.

Discussion on " White " Cattle.

With the above details available, it is now fitting that we should consider the position in Africa. First of all the colour pattern referred to as " sidet " by the Norwegians is seen throughout Africa in native cattle types with the exception of the Afrikander. According to Mr. F. N. Bonsma (personal communication of 20.5.36) who is investigating the genetics of Afrikander coloration the range varies from dark red, through blood red, light red, golden yellow, light yellow to white.

As described by Professor Isaachsen, the coloured side markings in South Africa also may be absent in which case the cattle are white, although the skin is not necessarily pigmented. The pigmentation varies from a uniform distribution to merely black points, *e.g.* teats, ears, muzzle and feet. In many cases on the body a flea-bitten appearance is seen. In Zululand the term " sidet " or sided is replaced by the word " Nkone " and a beast may therefore be either a black or red " Nkone ". If there is no marked colour, *e.g.* just a few grey hairs (speckled) or where the animal is " decolored " or white, it is said to be a " nyoniaipumuli ". This term, however, probably arose [according to letter 13/327(2) of 5.10.35 from Director of Native Agriculture to Director of Veterinary Services] from the fact that such cattle were confiscated by the Zulu chief Cetewayo, since " nyoniaipumuli " means " the bird that does not rest ". Although there seems to be a relationship between " nkone " and " nyoniaipumuli " coloration, only the former pattern appeals to the native, being recognised as a dominant colour and popular as far back as the days of Dingaan. It was this colour that was selected by Curson when visiting Zululand in December, 1935, with a view to purchasing Zulu cattle for a herd that has now been established at the Veterinary Research Laboratories, Onderstepoort.

As is evident in the Afrikander, white need not necessarily be associated with a prior "sidet" pattern, but may exist independently.

*Jamaica and Trinidad.**

Since in the Report on Cattle Breeding in the above islands Hammond (1932) describes certain matters of interest also to veterinarians in Africa, attention is drawn to the fact that in Jamaica four problems were investigated. These were:—

(*a*) The characters of the native stock and their capabilities of improvement by selection.

(*b*) The changes occurring in European breeds after their introduction.

(*c*) The characters and suitability of the Indian (Zebu) breeds, and

(*d*) Methods of cross-breeding adopted.

In regard to (*b*) he states "there could be no doubt that degenerative changes were occurring. These appeared to be associated . . . with the feeding conditions", and "There could be little doubt that European stock . . . gradually lost their type under these conditions and required continual importations to maintain their form and constitution. The causes for the degenerative changes probably consist of a combination of factors, among which may be mentioned the following in order of importance:

(1) Sub-lethal infections of tick fever (*B. bigeminum*).

(2) Lack of concentrated feeds and too much roughage.

(3) Unwise selection of bulls.

(4) Inbreeding, and

(5) Disadvantage of long thick coat..

When it is explained that the native stock of the West Indies was imported originally from Europe and has in the course of a few centuries become adapted to tropical conditions, we in Africa should realise how much more fortunate we are in possessing cattle types which have not only existed on this Continent for thousands of years, but which have actually been evolved in Africa.

Brazil.

Thanks to the courtesy of the officials of the Department of Agriculture, Brazil, a reply to the Questionaire was received through the Department of External Affairs, Pretoria, which in turn had communicated with the British Embassy at Rio de Janeiro.

Of particular interest to the African cattle owner is the fact that in South America, especially in Brazil, are Crioulo cattle which possess a skull not unlike that of the Hamitic Longhorn type, while the horns resemble very closely the upright lyre-shaped horns of many Sanga cattle.

The cattle of Brazil were derived about the middle of th 16th century (1534) from chiefly Portugal and, as explained by Curson and Epstein (1934), Hamitic Longhorn stock was responsible not only for cattle of similar type, existing to-day in the Iberian Peninsula, but also partly (*i.e.* with the Lateral-horned Zebu or Afrikander) for the Sanga type best represented in South Africa.

In general conformation, however, there is a striking difference between the Crioulo and the lyre-horned Sanga, namely in the presence of a cervico-thoracic muscular hump which the Sanga acquired from its Afrikander progenitor. No photographs were available from Brazil in regard to the Crioulo, but the following facts contained in the reply from Rio de Janeiro are worth extracting:—" Of the several types or breeds (in Brazil) there are some which excel either by their qualities or by their numerical or geographical distribution, *e.g.* Caracu, Mocha, Crioulo and Curraleira. The Caracu and Mocha are carefully selected and bred by the Federal Government of the Sao Paulo State. The Crioulo type exists scattered all over the country from north to south. It is being preserved in many of the states in the hinterland outside the sphere of the exotic breeds which have been imported. The Curraleira type still includes an appreciable number of cattle. The other types evolved *locally* are now only of historical importance. In fact their numbers are gradually diminishing and there is an increasing tendency towards their entire disappearance owing to cross-breeding with European breeds and *Bos indicus*, *i.e.* Shorthorned Zebu ".

The other types just referred to are the descendants of cattle introduced centuries ago by the Portuguese from Portugal, Portuguese Guinea, and India, and which in the course of time have degenerated.

Of the cattle whose improvement is fostered by the State the Mocha is of interest to African workers, for it is stated by the Brazilian authorities to be a " variacao espontanea do tipo crioulo ". Da Costa (1933), however, believes that the yellow polled Mocha cattle are of African origin, having been exported to Brazil from Portuguese West Africa in the early days of the settlement. As he explains, a consignment would include polled cattle just as it would to-day.

Argentine.

The officials of the Argentine Department of Agriculture went to considerable trouble to furnish information regarding the cattle of their country, but in the present article use will be made only of the data bearing on Crioulo cattle. The many photographs accompanying their communication are not only interesting but useful. As before, correspondence was carried out through the Department of External Affairs and the British Embassy at Buenos Aires.

The following extracts concerning Crioulo cattle are of particular interest.

"*The Colonial Argentine Cattle.*—On the arrival of the Spaniards in the La Plata territories in the 16th century the following species did not yet exist: bovines, equines, goats, sheep and swine. These domesticated animals were introduced by the first Spanish conquistadores.

Originally the black Argentine "criollo" were the descendants of the cattle which were brought by Colón to the Antilles and which were afterwards brought to Peru where they multiplied in an amazing manner. In 1543 General Francisco Aguirre introduced some black cattle from Chile and Lower Peru into Northern Argentine.

The progeny of these were introduced in the littoral; the first lot consisting of seven cows and a bull was brought along by the expedition of Sanabria which landed on the coast of Brazil. It was transported to the bank of the Paraná, opposite the Rio Monday whence it was embarked on a raft, which was run up the Paraná as far as its confluence with the Rio Paraguay at Asunción where it was landed in 1554.

This first settlement of black cattle was shipped in the port of San Lucas by the pioneer planter of the Rio de la Plata, Don Juan de Sanabria. These animals belonged to the Andalusian breed and were the origin of the present-day riches of Paraguay and of the Rio de la Plata.

In 1573 there was introduced in Santa Fé by Don Juan de Garay another lot of black cattle, from the herd brought by General Cáceres from the estancias of the pioneer of the Rió de la Plata, Don Juan Ortiz de Zárate.

The Black 'Criollo" Breed descended from the Andalusians.

The black cattle brought by the pioneers of the Rió de la Plata belong to the Andalusian or Iberian breed of Sanson. Its zootechnical characteristics are as follows: osseous system greatly developed, large head and large horns, heavily built and very moderate eater. Its hide is variegated, coloured light, light brown or dark. It gives very little milk, but is useful as a producer of meat and for work.

Zootechnical Characteristics of the Black 'Criollo' Cattle.

From the Andalusian cattle described above are descended the black 'criollo' cattle which present a few variations as regards their zootechnical characteristics resulting from the variety of circumstances, which have contributed to produce the 'criollo" variety which during the long colonial period has been one of the principal sources of wealth of the country by its production of meat and leather.

The zootechnical characteristics of the black Argentine ‘ criollo ’ cattle are as follows: adapts itself easily and breeds well. Its free life on the open plains during many centuries has given it characteristics of its own; it has massive bones (inherent in animals which travel great distances), and owing to its life exposed to all the inclemencies of the weather its pelt is extraordinarily thick. It is of great rusticity and breeds and grows well on poor soil; it is even found in the hills and in the woods. It matures and fattens late, circumstances which detract from its economic value. The black ‘ criollo ’ attains its maximum development after seven or eight years. It is principally used as a slaughter beast, its meat is considered very palatable, but it is also used for work and to produce milk, although the daily yield is seldom more than 5 litres.

Present-day Types of Black ‘ Criollo ’ Cattle.

The ‘ criollo ’ cattle are still largely bred in many of the provinces where there is little change in the grazing or where it improves but slowly. It has been observed as a general rule that the ‘ criollo ’ cattle are slower in their development in proportion as the pasture improves. In the provinces in which the grazing is very poor, the ‘ criollo ’ is rickety and has diminished in height.

The pure ‘ criollos ’ and the quarterbreds which are used for canned meat and extract of meat are called ‘ tipo saladero ’ and ‘ tipo matadero ’ and yield on an average (clean meat): young bull 150 kilos or say 37 per cent.; cow 123 kilos or say 34 per cent. The weight of these animals on the hoof is as follows: young bulls 427 kilos and cows 357 kilos.

The ‘ criollo ’ cattle thrive well both on the plains and on the highlands, on the slopes of the hills, in shrub-country and in the forests. They are hardy and can thrive on but little food.

Since immemorial times they have been born and bred in country infected with ticks and they have acquired immunity from the bovine malaria known as ‘ tristeza ’ (melancholy). In the provinces of Santa Fé, Entre Rios, Corrientes, Córdoba and Salta and in the territories of the Chaco, Formosa and Misiones, where ticks transmit Anaplasmosis and Piroplasmosis, the ‘ criollo ’ cattle keep perfectly healthy whereas the imported European breeds die in great numbers ”.

The conformation of Crioulo cattle is seen in Figs. 86 and 87.

LITERATURE (^{37}).

BOHRMANN, A. H. L. (1924). Park Cattle. *Milk Recording.*

HAMMOND, J. (1932). *Report on cattle-breeding in Jamaica and Trinidad.* Empire Marketing Board 58. His Majesty's Stationery Office, London.

HIRSCH, S. (——). *Stock breeding and dairying in Syria.*

HOROWITZ, E. J. (——). *Breeds of cattle in Palestine.*

WALLACE, R. (1907). *Farm live stock of Great Britain.* Oliver and Boyd, 4th Edit.

MISCELLANEOUS. Leaflets of 1926. Breeds of cattle in the Bombay Presidency. Dept. of Agr., Bombay.

I. The Kankrej Breed of Gujerat.
II. The Gir Breed of Cattle.
III. The Sindhi or Red Karachi Cattle.
IV. The Krishna Valley Breed.
V. The Khillari Breed of Cattle.
VI. The Thar Parkar Breed.

CHAPTER IV.

GENERAL CONCLUSION.

Since the Questionaire dealt essentially with African cattle and only incidentally with cattle in certain countries outside Africa, it seems advisable to conclude by referring firstly to the position within Africa and secondly to the position without Africa.

(*a*) AFRICAN CATTLE.

(1) While the replies to the Questionaire provide a proportion of the data presented, it is manifest that the scope of the investigation was extended chiefly by including territories not originally circularised. In the vast majority of cases it was the veterinary officials who furnished the particulars required(54).

(2) Owing to the interval which has elapsed between the issue of the Questionaire and the completion of this compilation, much light has been thrown upon the subject of African Cattle Husbandry generally, *e.g.* origin, conformation and classification. This recent information accordingly rules out certain questions which were asked in 1931. A striking example is the territorial distribution of cattle types, a description of which was given in a paper at the 1935 (September) meeting of the South African Veterinary Medical Association [see *Jl. S.A. Vet. Med. Assn.* VII (1) 1936].

(3) It was realised that the incorporation of information bearing on environmental features and production would be unsatisfactory. Not only were the replies in some instances silent in this respect, but in others, the details were exceedingly meagre.

(4) Accordingly it was felt that it would be best to concentrate upon a description of the cattle types and their distribution, but where some feature referred to in the previous paragraph had received special attention, *e.g.* milk production by Anderson in Kenya, it was included.

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(54) Although entirely outside the scope of this investigation, it is noteworthy from the replies that the veterinarians trained in Latin-speaking countries possess generally a more profound knowledge of Animal Husbandry (at least from an academic aspect) than those educated in English-speaking countries. Recent alterations in the curriculum of the British veterinary colleges in regard to this point are significant.

(5) Of the several methods possible to describe the distribution of the several types, it was decided to take the logical, namely the territories occurring along the various migration routes in their chronological order.

(6) Where territories have been omitted, *e.g.* Italian and Spanish colonies, this is due to the absence of details, although in most cases it is possible to deduce the state of affairs.

(7) There is little doubt but that originally all cattle, except the ancient Hamitic Longhorn of Egypt, came from Asia. All migrations passed through North-East Africa, chiefly Egypt, except the Shorthorned Zebu which was also introduced along the east coast of Africa as far South as the Zambesi River.

(8) The probable migration paths *to* and *in* Africa and the likely periods this occurred are shown in Map I.

(9) The present approximate distribution is shown in Map II, due allowance having been made for Glossina and desert regions, *e.g.* Sahara desert.

(10) Of still greater importance would be a map indicating the approximate numerical distribution of cattle such as appearing in Bosman's *Cattle Farming in South Africa*(55). In this case a dot represents 1,000 head of cattle in his Map I.

(11) Apart from the distribution having been indicated in Map II, the position has been summarised at the end of the description of each migration, *e.g.* pp. 644-645 and 672-674.

(12) Although only of secondary consideration the information presented in the contribution may be helpful to anthropologists. Dart (1933) states that " There is no more vital aspect of anthropology than the study of domestic animals ". It is therefore hoped from an anthropological aspect that the evidence afforded by the migration routes and present distribution will be at least as valuable as that derived from other branches of science, particularly the study of human types including cranial measurements, blood groups, languages, customs, utensils, etc.

(13) A striking fact is that the French authorities possess excellent text-books on the livestock of their colonies, whereas such information concerning British territories is scattered throughout annual reports.

(14) Investigation is required regarding the distribution of cattle types in West Africa and upon such matters as degeneration of horns (unassociated with grading up with Brachyceros) as described along the littoral of French Dahomey and in the Transkei, Union of South Africa (Thompson, 1932), genetics of coloration, hump, etc.

(15) It is noteworthy that no veterinary department exists in either Gambia or Sierra Leone.

(55) Being South African Agricultural Series, No. 10, and published by the Central News Agency in 1932.

(16) This survey indicates that in addition to the three parent stocks (Hamitic Longhorn, Brachyceros and Lateral-horned Zebu) and the three derived types (Shorthorned Zebu, Sanga and Lyre-horned Zebu), there are possibly two other distinct derived types. These are both in West Africa and result from the intermixture of Brachyceros and Hamitic Longhorn in the one case (Mandingo), and Brachyceros and the thoracic humped Zebu in the other.

(17) Finally, the photographs reproduced constitute the most comprehensive set yet published.

(*b*) EXTRA-AFRICAN CATTLE.

Although the original intention of the Questionaire was to investigate the relationship of certain African and extra-African cattle, based on conformation or coloration, recent independent observations, chiefly by Epstein, rule out the necessity of proceeding in this direction. As a matter of interest, however, a résumé has been given of the position in the countries concerned.

APPENDIX.

Cattle populations of the various African territories(56):—

Territory.		Approximate No. of Cattle.	Authority.
	Egypt	776,000	Le Plae (1933).
French North Africa	Tunis	502,000	"
	Algeria	937,000	"
	Morocco	2,092,000	"
French West Africa	Upper Volta Mauritania Niger	(1,576,000)	"
	Sudan	(1,138,000)	"
	Senegal	(393,000)	"
	Guinea	—	
	Ivory Coast	—	
	Dahomey	—	
	TOTAL for French West Africa	3,352,512	French Minister, Pretoria. Letter of 13.5.36 (for 1934).
	Gambia	—	
	Sierra Leone	—	
	Gold Coast	192,000	*Ann. Vety. Rpt.*, 1934-35.
	Nigeria	2,675,961	*Ann. Vety. Rpt.*, 1933.
	Portuguese Guinea	62,000	Da Costa (1932).
French Equatorial Africa	Chad	1,451,000	Le Plae (1933).
	Cameroons	645,000	"
	Gabon Middle Congo Ubangi Shari	4,000	"
	Abyssinia	15,000,000 (?)	*Encyclopaedia Britannica.*
	Anglo-Egyptian Sudan	1,300,000	Le Plae (1933).
	Uganda	2,151,000	*Ann. Vety. Rpt.*, 1934.
	Kenya	5,191,000	Le Plae (1933).
	Tanganyika	5,170,162	*Ann. Vety. Rpt.*, 1930.
	Belgian Congo	1,300,000	Le Plae (1933).
	Angola	1,500,000	Da Costa (1933).
	Northern Rhodesia	522,693	*Ann. Vety. Rpt.*, 1934.
	Nyasaland	190,381	" "
	South-West Africa Protectorate	854,899	Letter of 3.7.36 from S.V.O., Windhoek (for 1935).
	Bechuanaland Protectorate	1,300,000	Letter of 18.6.36 from C.V.O., Mafeking (for 1934).
	Basutoland	350,000	—
	Swaziland	450,000	—
	Southern Rhodesia	2,716,762	*Ann. Vety. Rpt.*, 1934.
	Mozambique	519,149	Botelho (1933).
	Union of South Africa	9,986,630	Min. of 9.7.36 from Director of Census (figures for 31.8.34).
		(57)	

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(56) In addition to the blanks below, no details are available with regard to territories not included in the Appendix, *e.g.* Spanish Morocco, Rio de Oro Spanish Guinea, Liberia, Libya, Eritrea, and both British and Italian Somaliland.

(57) See footnote 17.

ILLUSTRATIONS.*

Egypt.

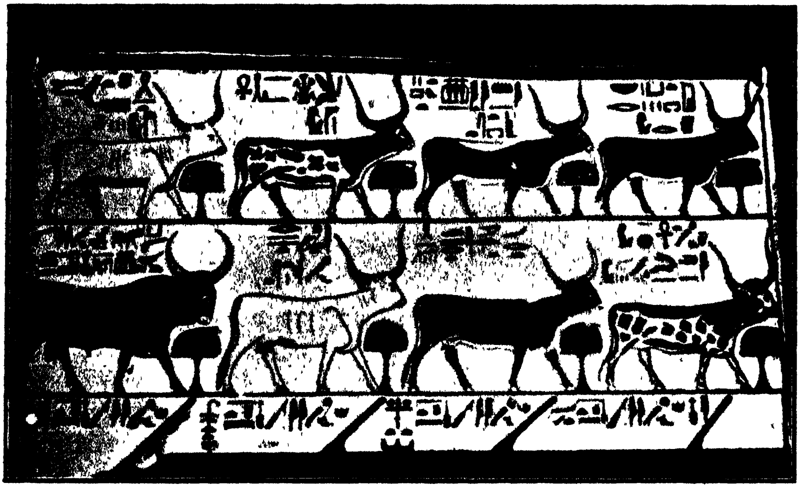

Fig. 1.* Original Hamitic Longhorn cattle.

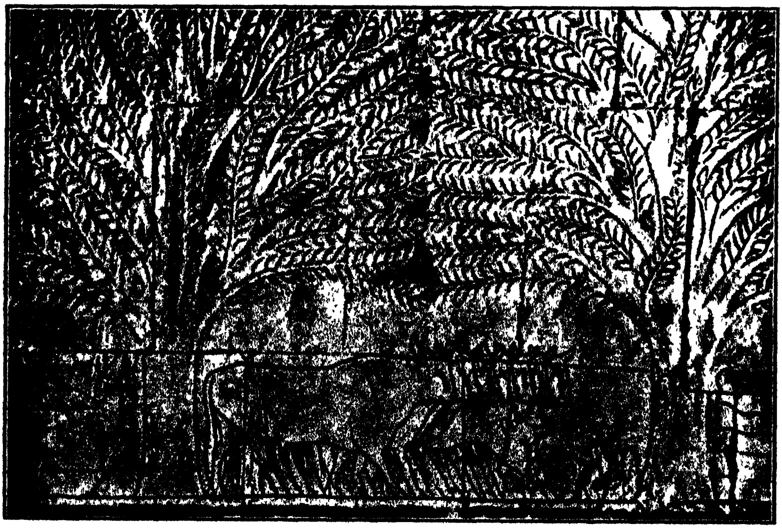

Fig. 2.* Ancient cattle of Brachyceros type.

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* Photographs received along with replies to Questionnaire. The source (either photographer or sender) in *other* cases is indicated by name.

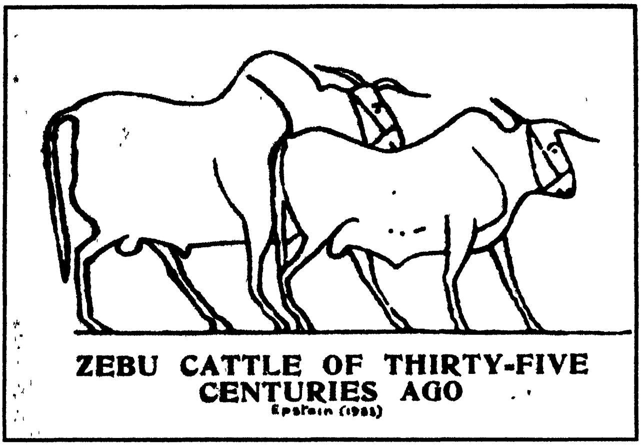
ZEBU CATTLE OF THIRTY-FIVE CENTURIES AGO
Epstein

Fig. 3. The original Longhorned Zebu (now Afrikander). (Stegmann v. Pritzwald, 1924.)

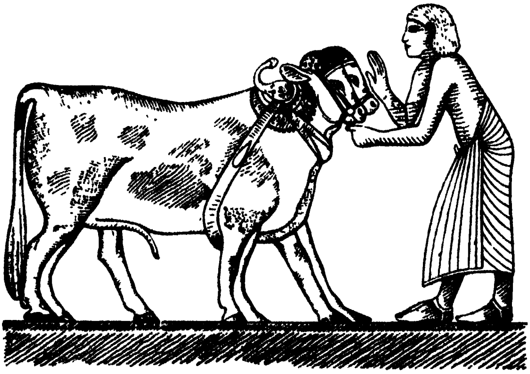

Fig. 4. A polled bull of ancient Egypt. (Kronacher, 1921.)

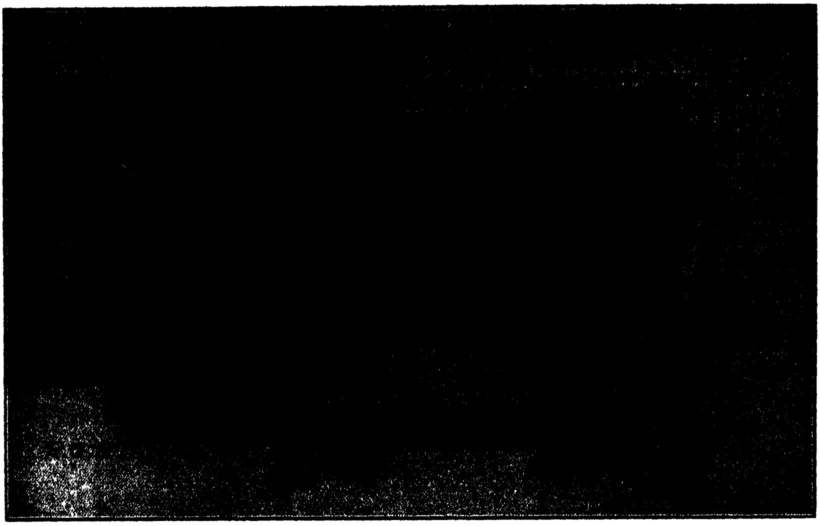

Fig. 5.* Damietta bull, Lower Egypt.

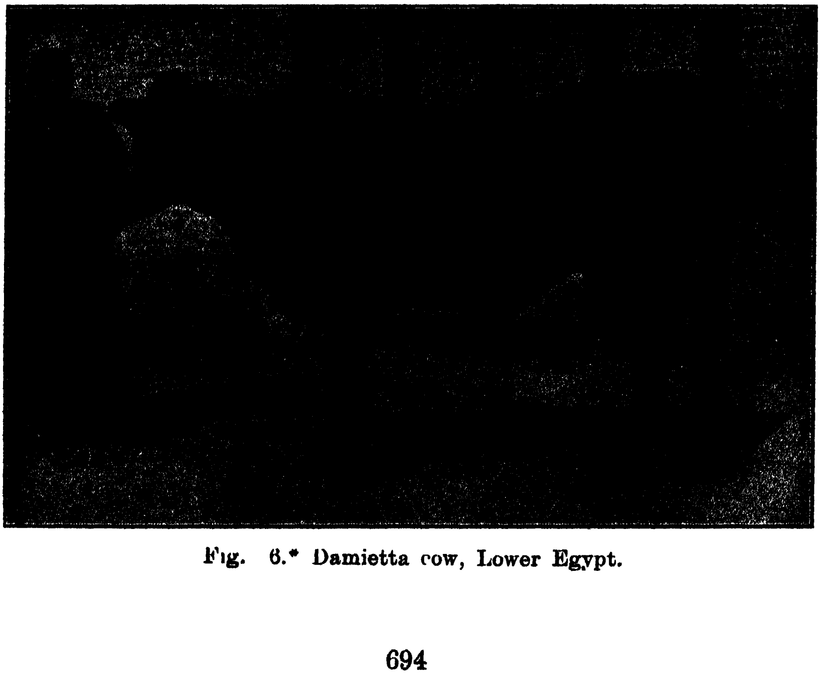

Fig. 6.* Damietta cow, Lower Egypt.

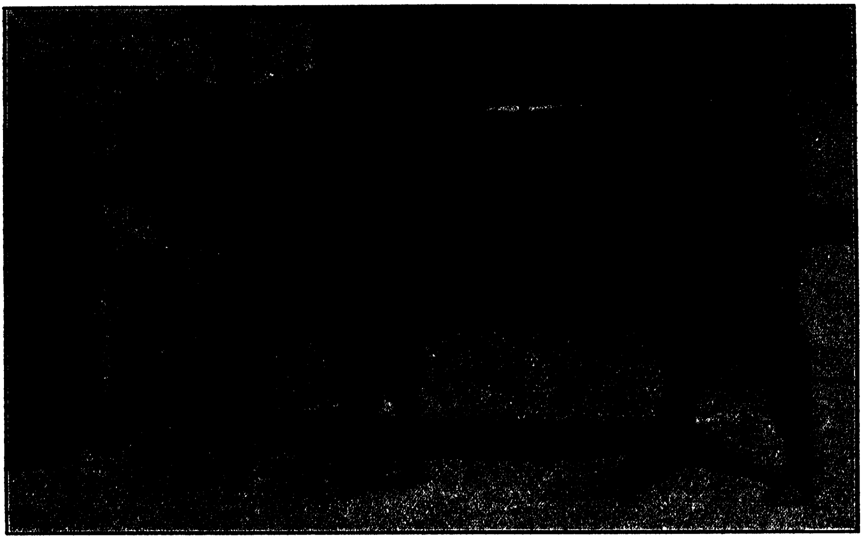

Fig. 7.* Baladi bull, Lower Egypt.

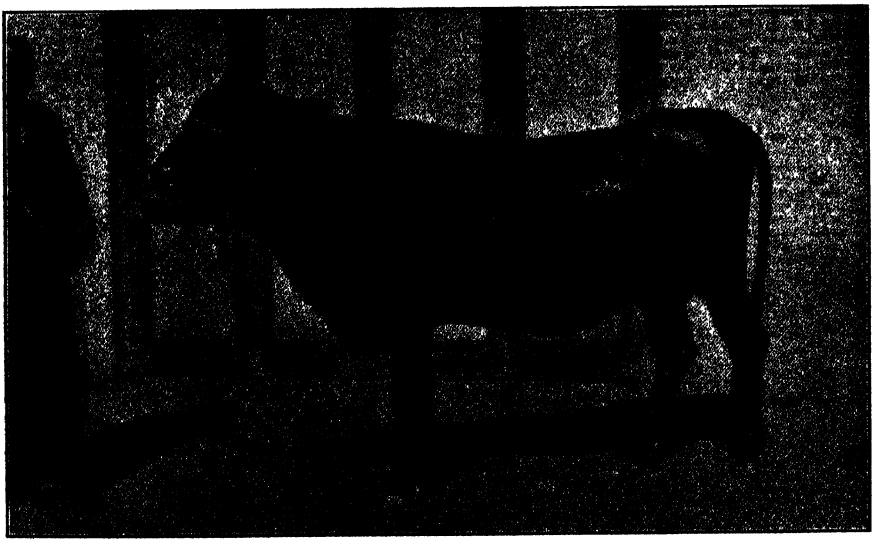

Fig. 8.* Baladi cow, Lower Egypt.

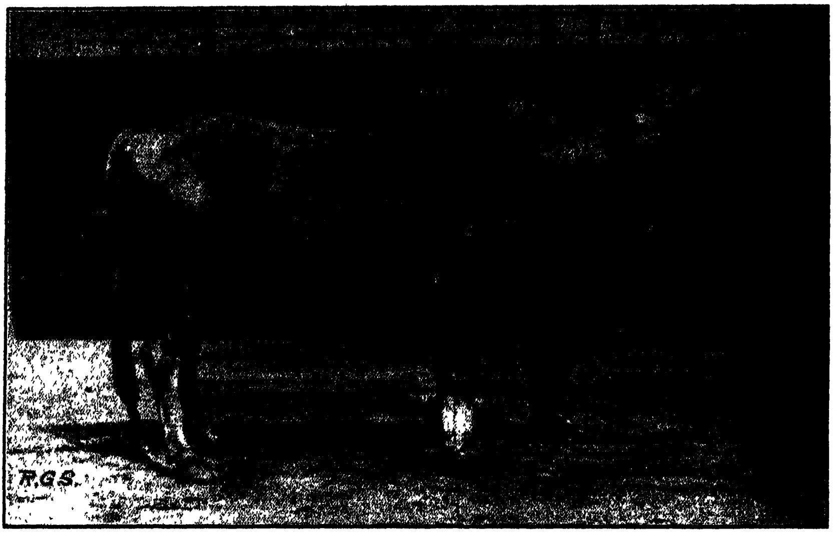

Fig. 9.* Saiidi bull, Upper Egypt.

Fig. 10.* Saiidi cow, Upper Egypt.

Fig. 11.* Marriouti bull of the desert

Fig. 12.* Marriouti cow of the desert

FRENCH NORTH AFRICA.

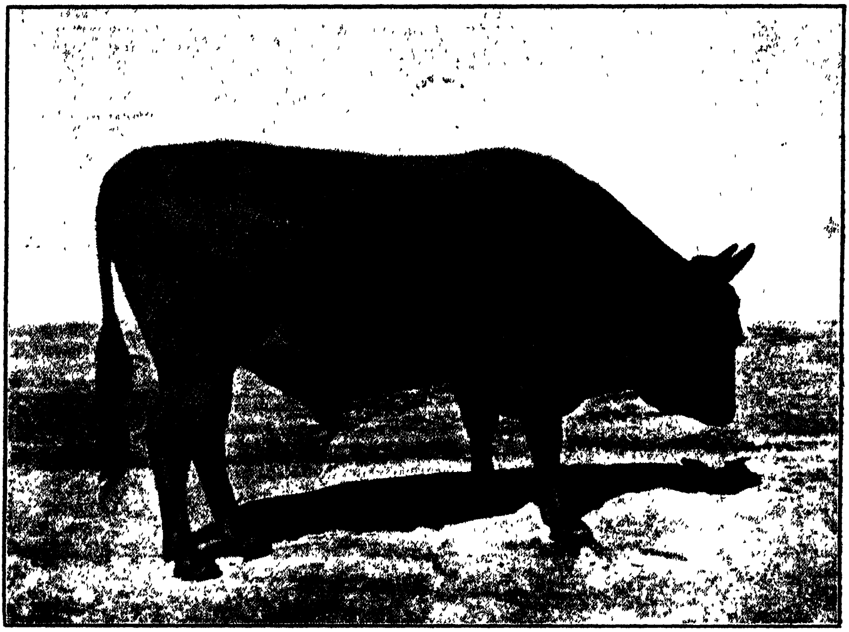

Fig. 13. Tunisian bull [Saint-Hilaire, H. G. (1919), Plate XIX].

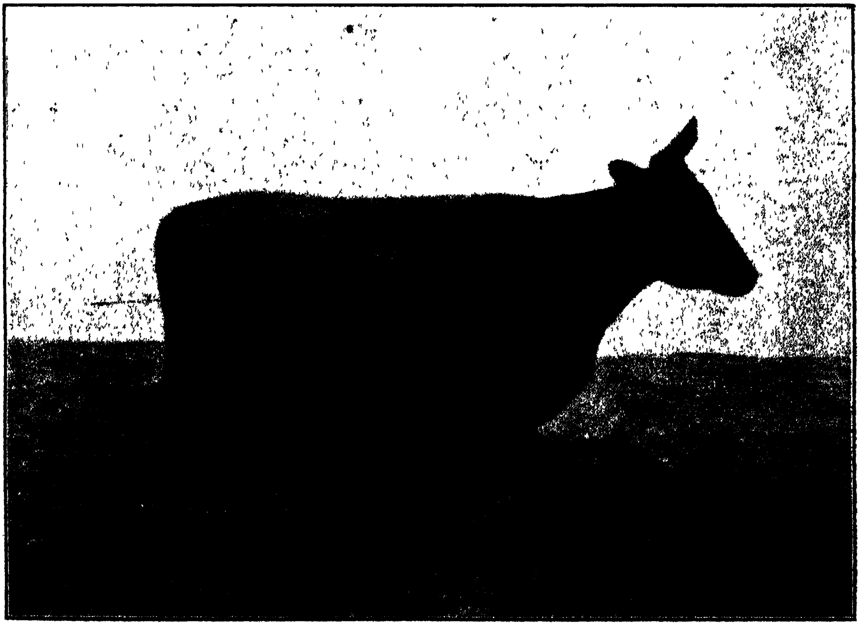

Fig. 14. Tunisian cow [Saint-Hilaire, H. G. (1919), Plate XIX.]

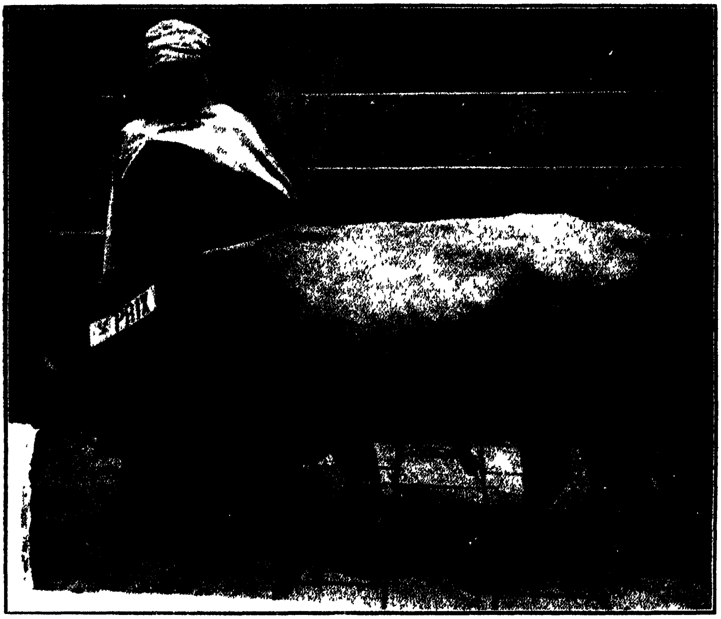

Fig. 15.* Algerian Cheurfa bull (Service de l'Elevage du Constantine).

Fig. 16. Algerian Guelma bull [Saint Hilaire, H G. (1919), Plate XVIII].

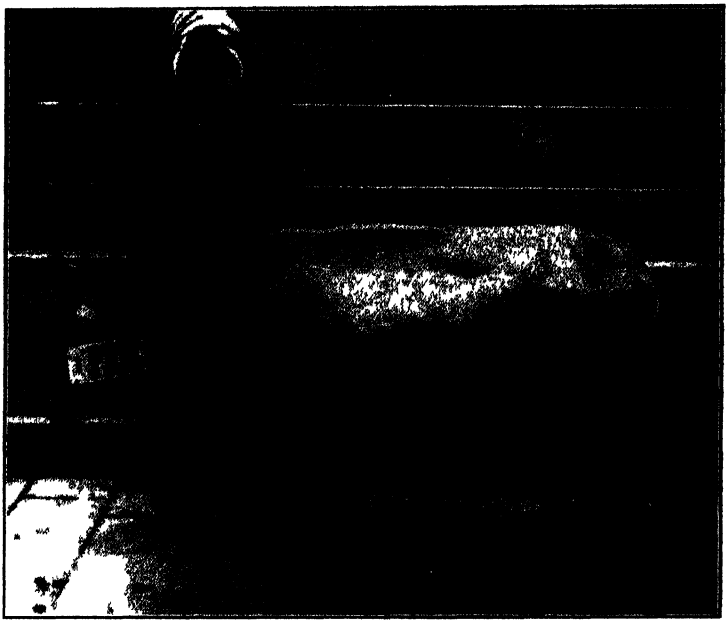

Fig. 17 * Algerian Guelma cow (Service de l'Elevage du Constantine)

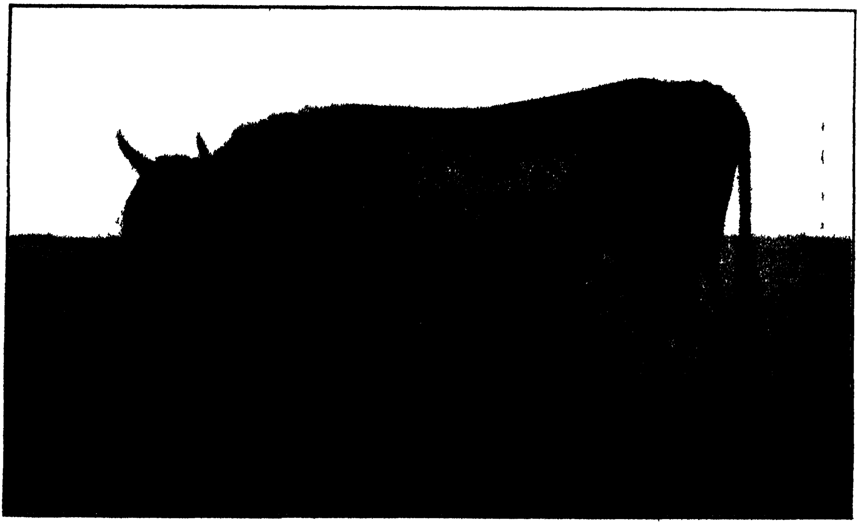

Fig. 18. Moroccan bull [Saint-Hilaire, H. G. (1919), Plate XXI].

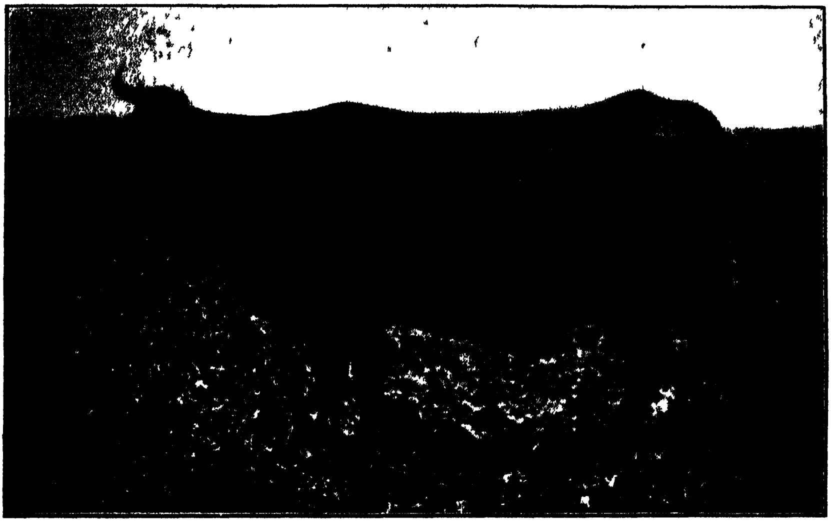

Fig 19. Moroccan cow [Saint-Hilaire, H G (1919), Plate XXI].

FRENCH WEST AFRICA (²)

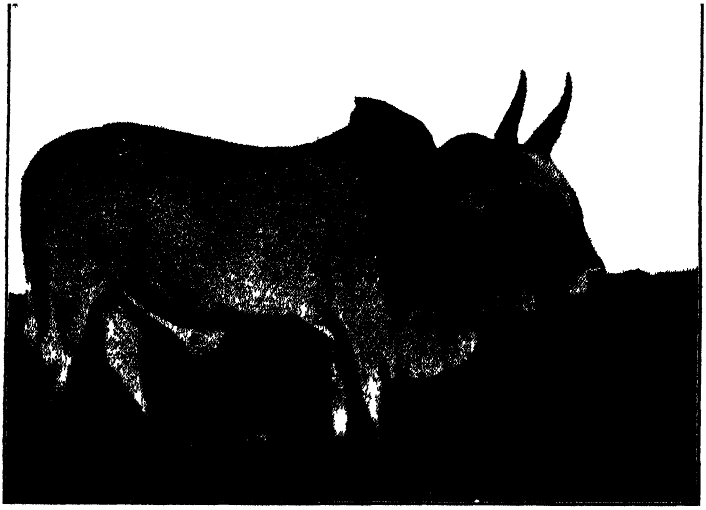

Fig. 20. The Peulhe or Gobra Zebu (Lyre-horned Zebu) Notice the thoracic hump and lyre-shaped horns. The appearance strongly suggests Hamitic Longhorn influence. (Pierre, Plate X.)

(²) As Brachyceros cattle are shown in Figs. 13-19, there is no need to illustrate the type as occurring in West Africa. See Epstein (1934), Pierre (1906), and Henderson (1929).

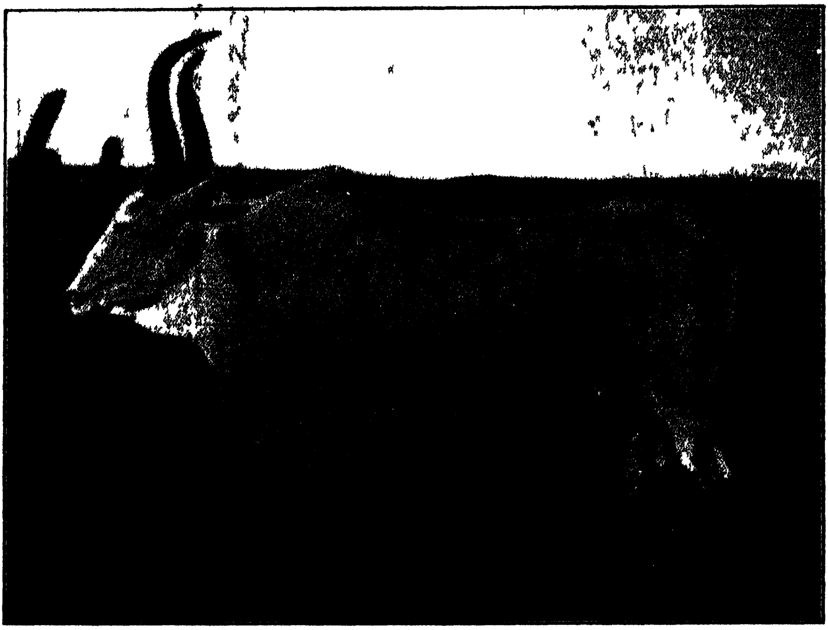

Fig 21 The Moorish or Gabaruyé Zebu (Lyre-horned), possessing the same general features of the Peulhe Zebu (Pierre, Plate X)

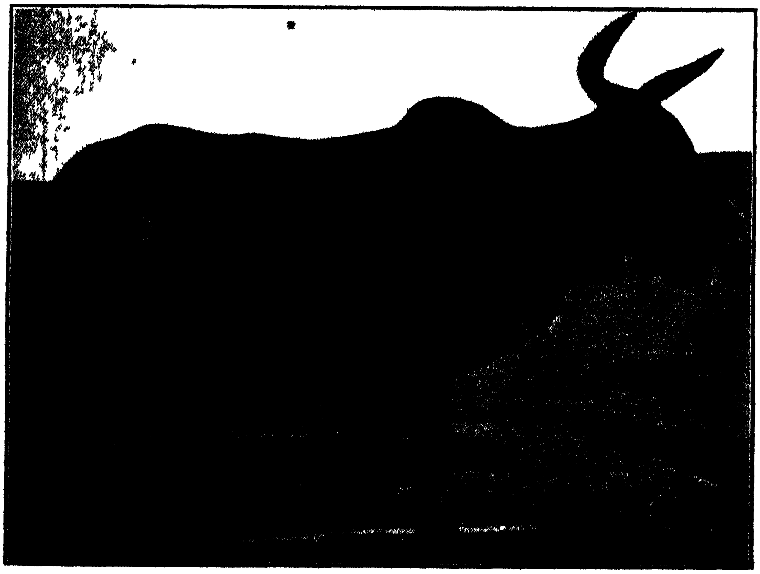

Fig. 22 The Nigerian or Foulbé Zebu The Hamitic Longhorn features are less evident than in Figs 20 and 21. (Pierre, Plate XI)

Fig. 23. The Fogha Zebu, apparently the sub-type showing most markedly the characteristics generally recognised as those of the Short-horned Zebu. (Pierre, Plate XIV.)

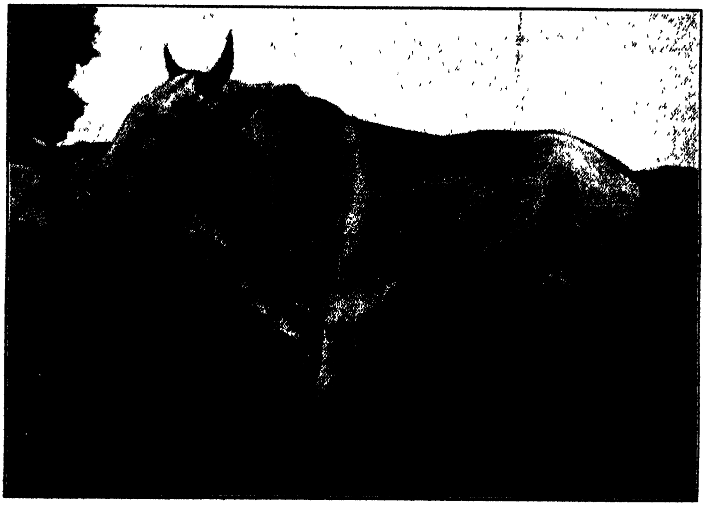

Fig. 24. A Bambara or Mandé bull, described by Pierre as resulting from the Zebu-Brachyceros cross but apparently a representative of the Sanga type. (Pierre, Plate XIII.) Notice the cervico-thoracic hump.

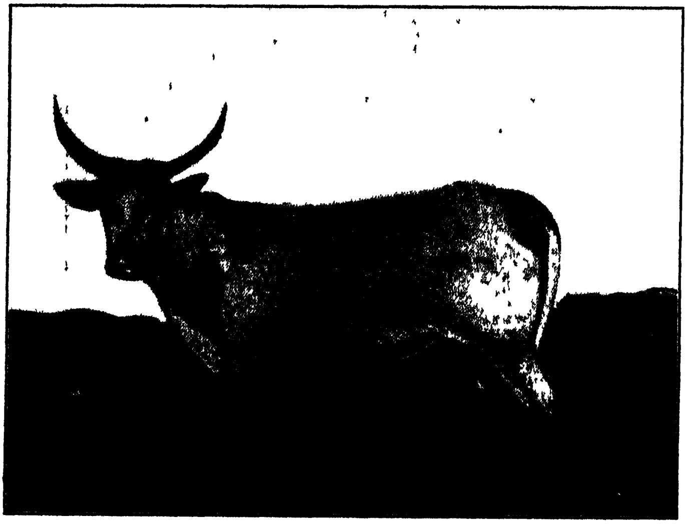

Fig. 25. A Bambara or Mandé cow Notice the cervico-thoracic hump of the Sanga. (Pierre, Plate XIII.)

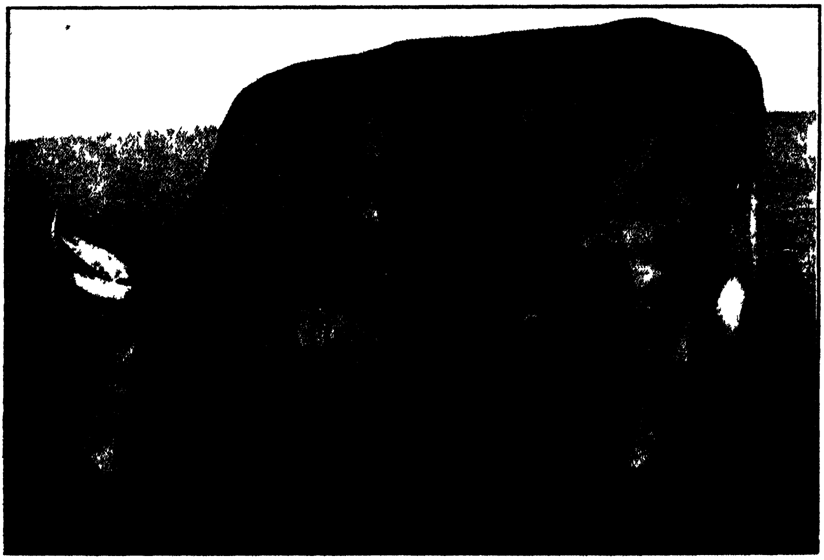

Fig. 26. A Djakoré or Senegalese ox which resembles very closely many Sanga cattle, *e.g.* Bechuana. Through the head being lowered the cervico-thoracic hump is not evident. (Pierre considers this a cross between the Zebu and Brachyceros). (Pierre, Plate XIV.)

LIBERIA

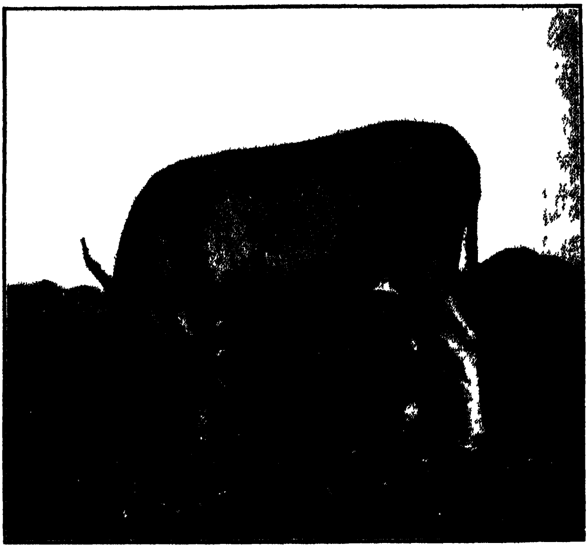

Fig 27 A Mandingo cow of West Africa (Fig 323 Johnston) This figure suggests a beast of Hamitic Longhorn origin, *i e* assuming there is no hump

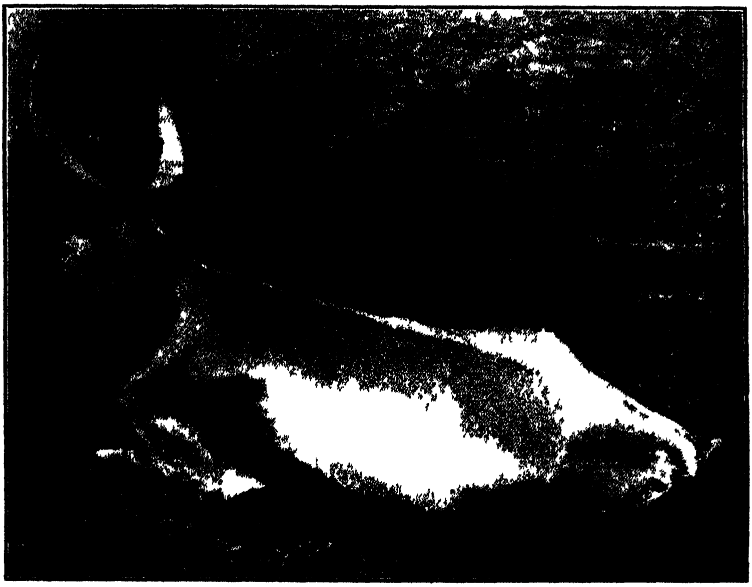

Fig 28. Another view of a Mandingo cow, Liberia (Fig 326, Johnston) The horns are long and lyre-shaped and there is no hump

GOLD COAST

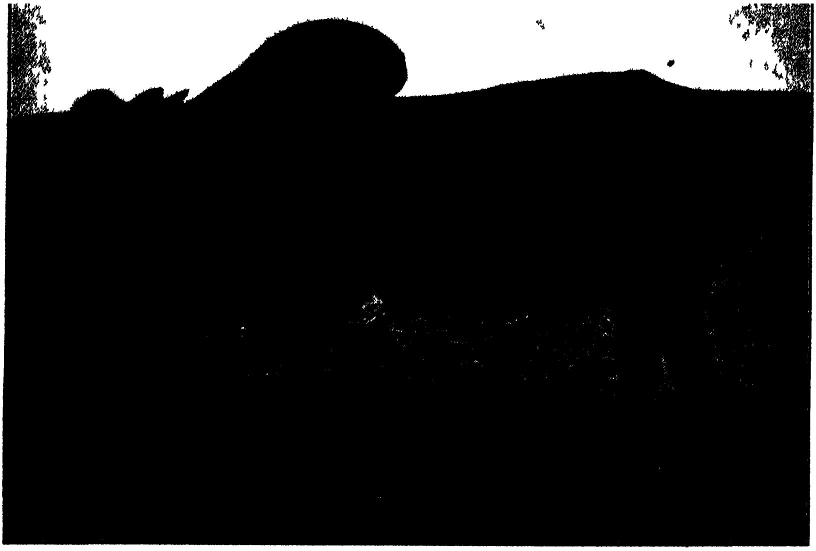

Fig. 29. Zebu bull, Gold Coast. Horns are not typical either of Lateral-horned or Shorthorned Zebu. (*Annual Report, Veterinary Department* 1929-30 Gold Coast.)

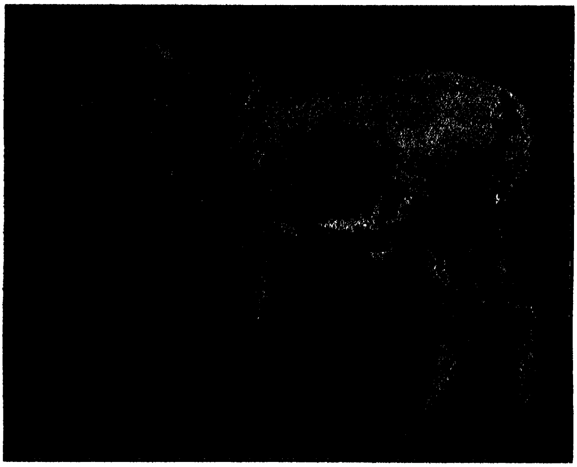

Fig. 30. A calf resulting from the cross between a Shorthorned Zebu bull and Brachyceros cow. Notice the hump, the situation and nature of which it is difficult to determine from the illustration. (*Annual Report, Veterinary Department*, 1929-30. Gold Coast.)

NIGERIA.

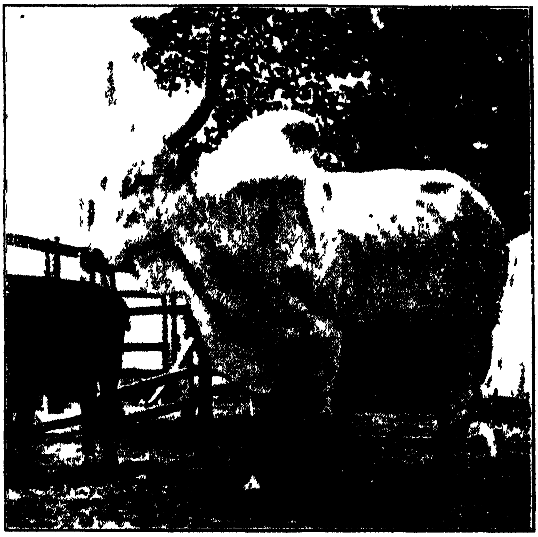

Fig. 31. The Lyre-horned Zebu of Nigeria Notice the thoracic and musculo-fatty hump, lyre-shaped horns and well-marked dewlap (*Annual Report, Veterinary Department*, 1926.)

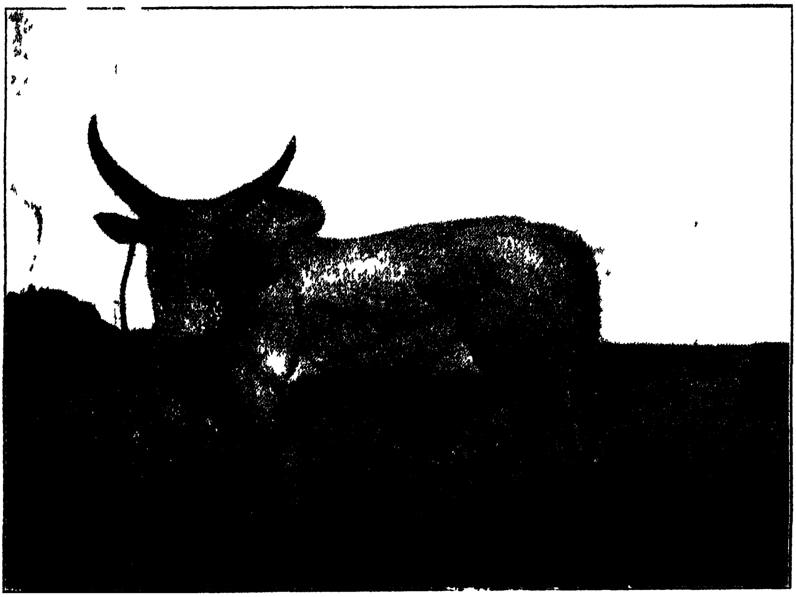

Fig. 32. A White Fulani (Lyre-horned Zebu) bull photographed at Maidontoro near Vom on 12.1.1927. (Dr. P. J. du Toit.)

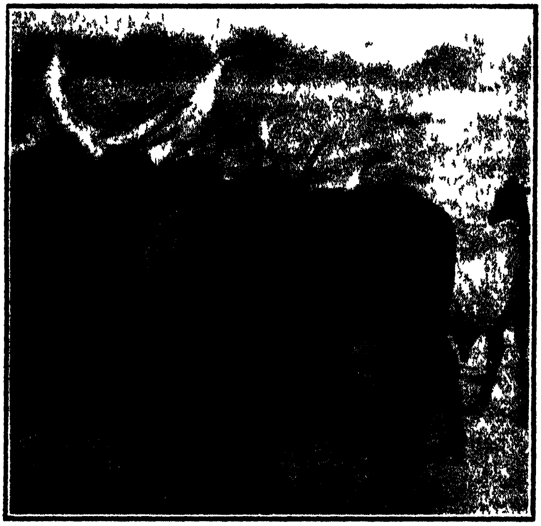

Fig. 33. Lyre-horned (Red Fulani) Zebu cattle photographed on road from Hadeija to Kano on 18.1.1927. (Dr. P. J. du Toit.)

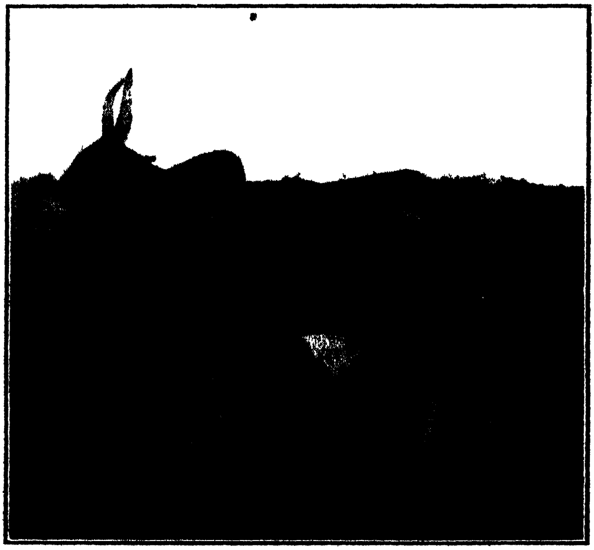

Fig. 34.* Lyre-horned Zebu, Sokoto-type, Nigeria.

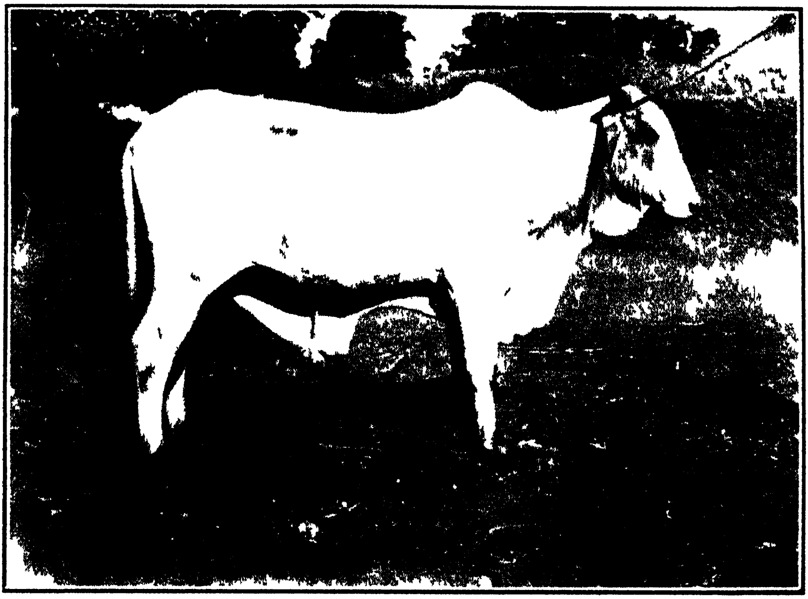

Fig 35 * Shorthorned Zebu bull

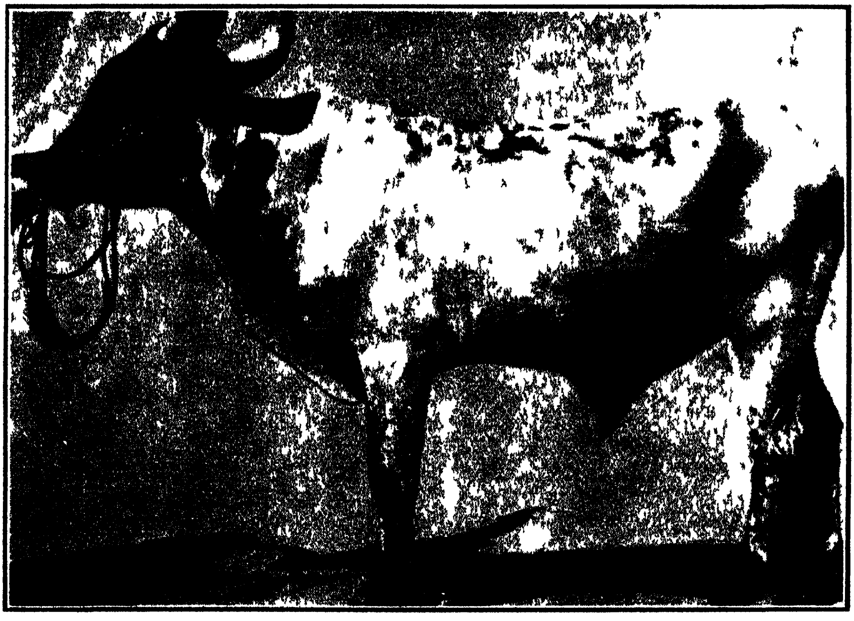

Fig 36 * Sanga ox, found near Lake Chad

UGANDA.

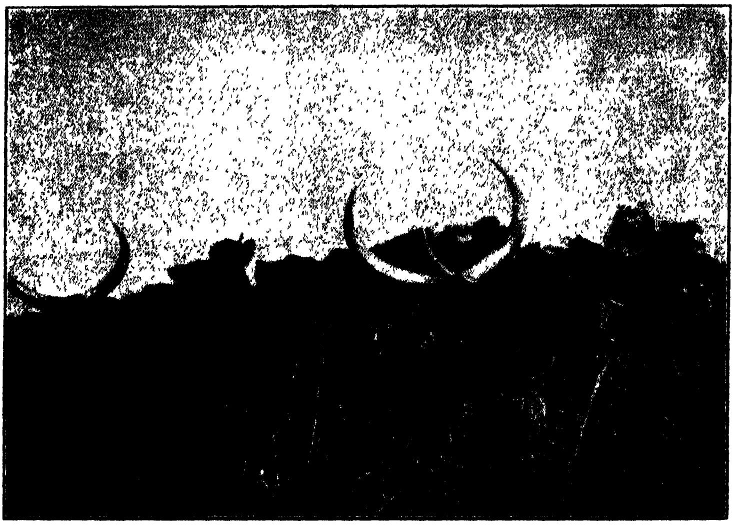

Fig. 37.* Red Ankole cattle at Koja Experimental Farm.

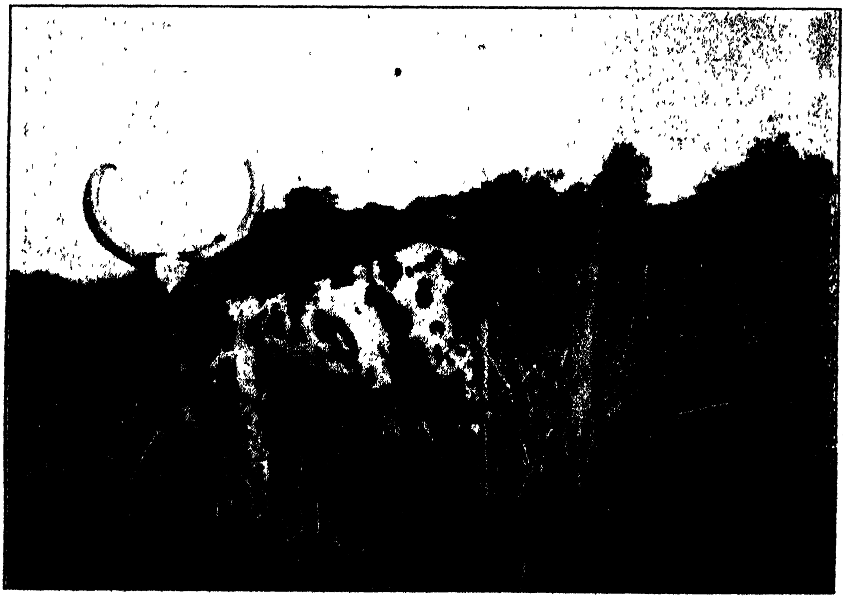

Fig. 38.* Typical Unyororo cattle of Sanga type at Koja Experiment Farm.

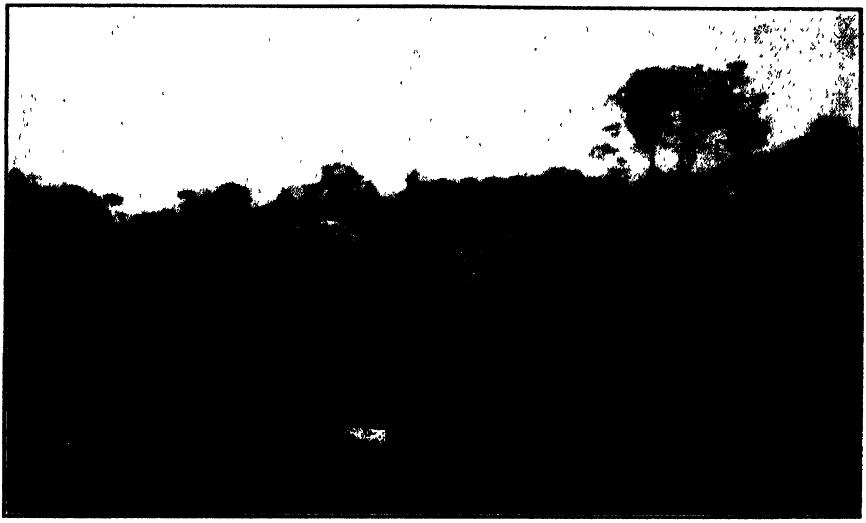

Fig. 39.* A typical Shorthorned Zebu bull from Eastern Province.

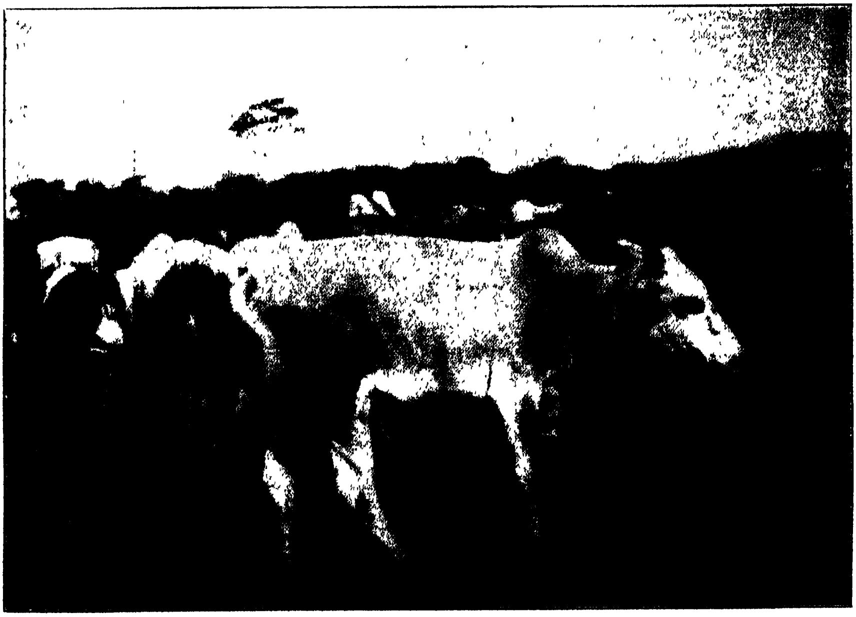

Fig. 40.* A typical Shorthorned Zebu cow from Eastern Province.

KENYA.

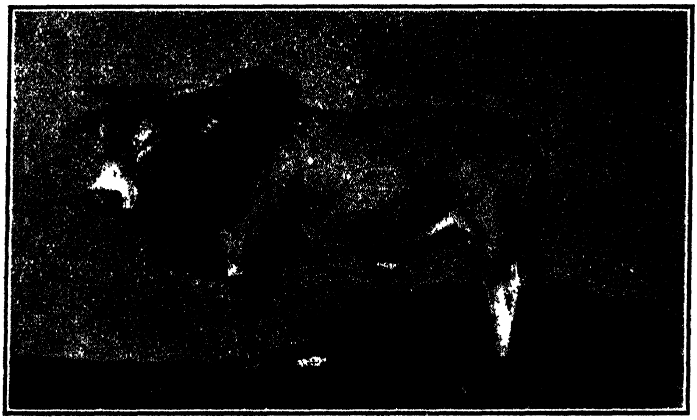

Fig. 41.* Nandi bull, red

Fig. 42.* Nandi cow.

Fig. 43. North Masai bull from Laikipia. (J. R. Hudson, M.R.C.V.S.)

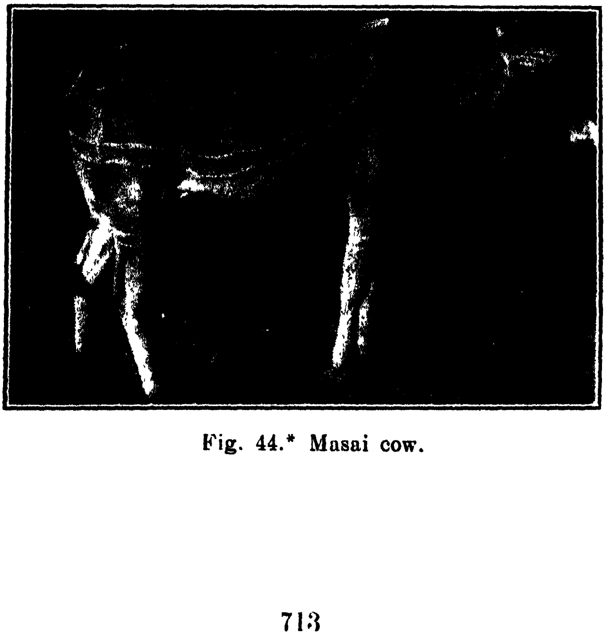

Fig. 44.* Masai cow.

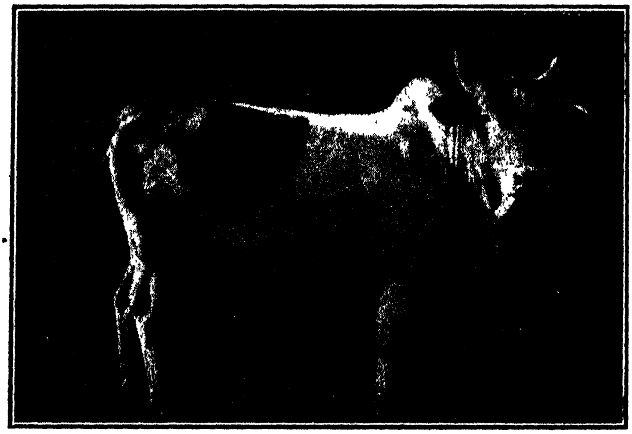

Fig. 45.* Akamba cow

BELGIAN CONGO

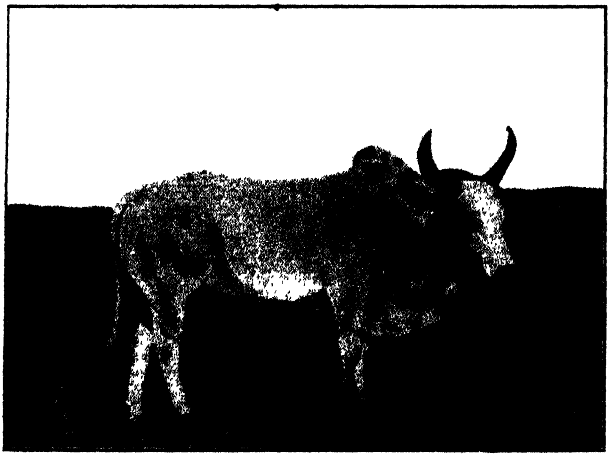

Fig. 46. Wadai-Dinka bull. Taken from Le Plae's *Organisation et Exploitation des Elevages au Congo Belge* (1933), Fig. 32, p. 91.

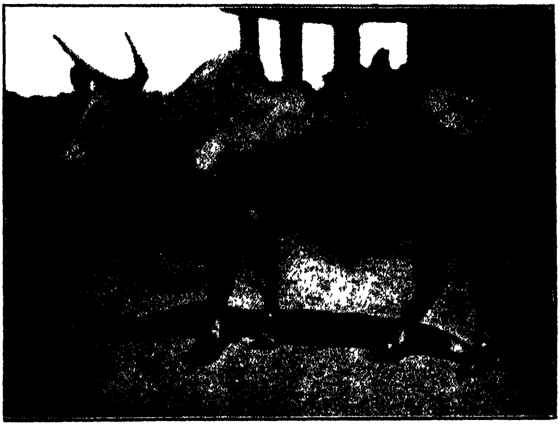

Fig. 47. Wadai-Dinka bull. Taken from the same source, Fig. 33, p. 91. Note in each case the short broad forehead and the cervico-thoracic hump.

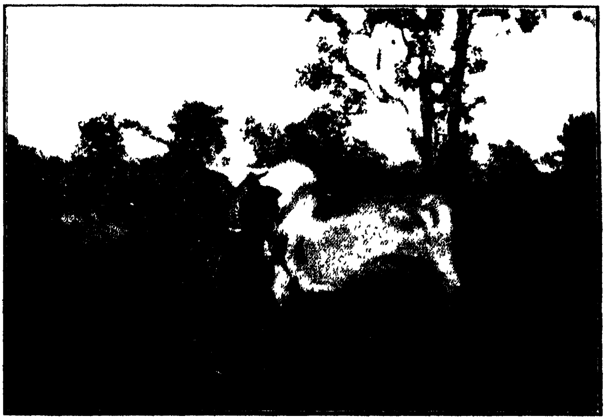

Fig. 48. A Lugwaret bull taken from Le Plae's work, being Fig 40, p. 99. Observe thoracic hump.

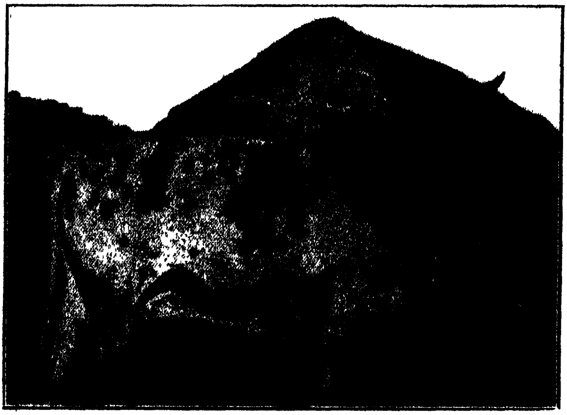

Fig. 49. Cow of Sanga type from Chief Blukwa's herd (west of Lake Albert). Le Plae, Fig. 26, p. 82. Apart from the dewlap, which is well developed due to Shorthorned Zebu influence, the cow is no different in conformation to Sanga cattle in South Africa.

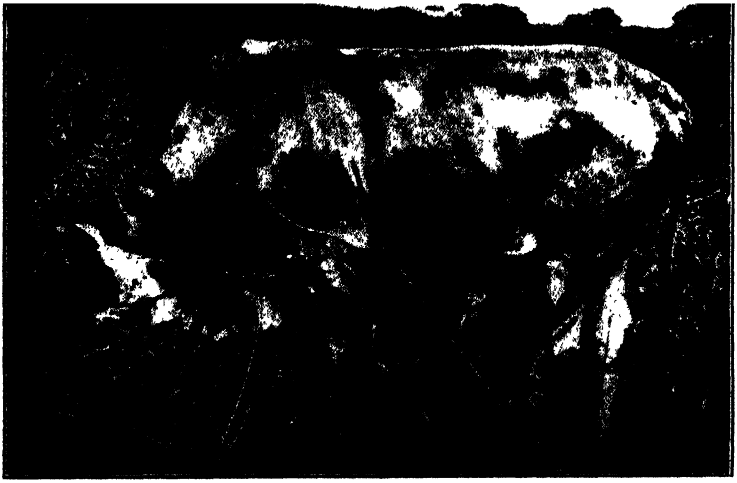

Fig 50 Kivu (Sanga) bull. polled. From Carlier's paper " L'Elevage au Kivu " (*Bull. Agric. du Congo Belge*, Sept., 1912, Fig. 516, p. 753).

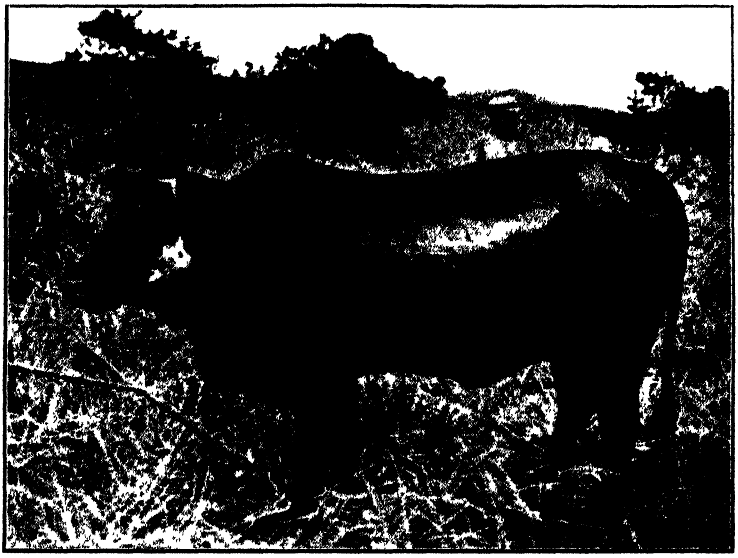

Fig. 51. Kivu (Sanga) cow, polled. From same source, Fig. 515, p. 753.

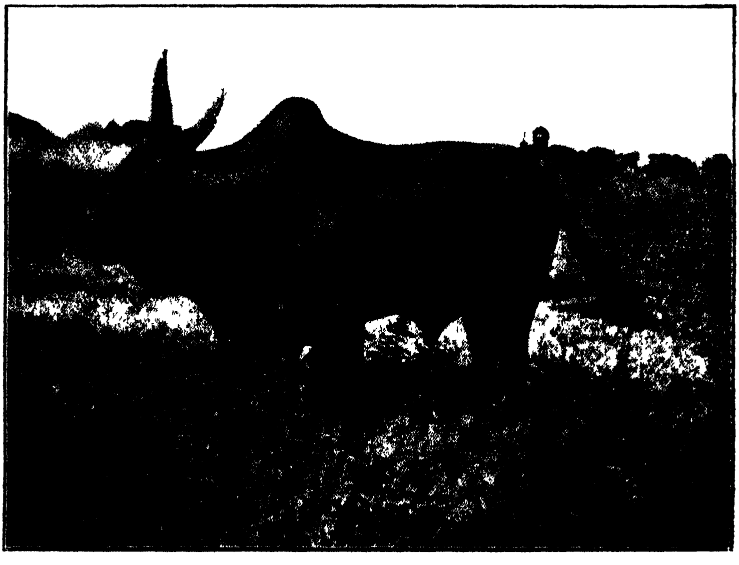

Fig. 52. Kivu (Sanga) bull, with medium horns. Although of Sanga type there is an admixture of Shorthorned Zebu judging from profile. From Carlier's paper in the December, 1912, issue of the *Bull. Agric. du Congo Belge*, Fig. 528.

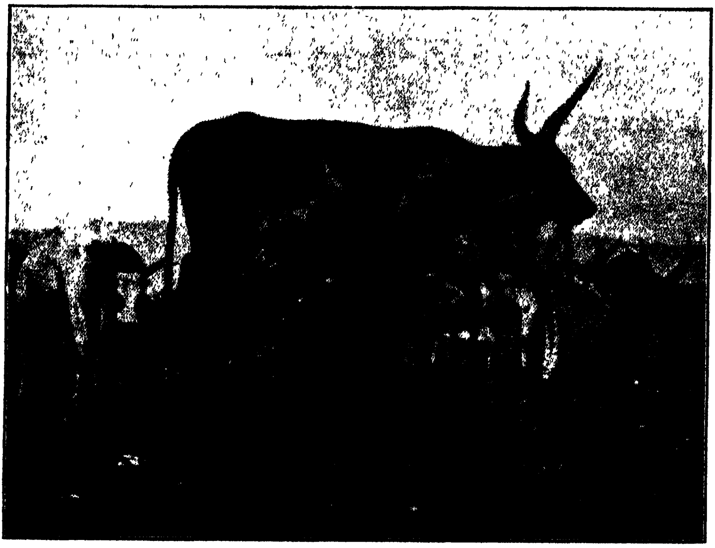

Fig. 53. Kivu (Sanga) cow, with medium horns From *Bull. Agr. du Congo Belge*, Dec., 1912, Fig. 529.

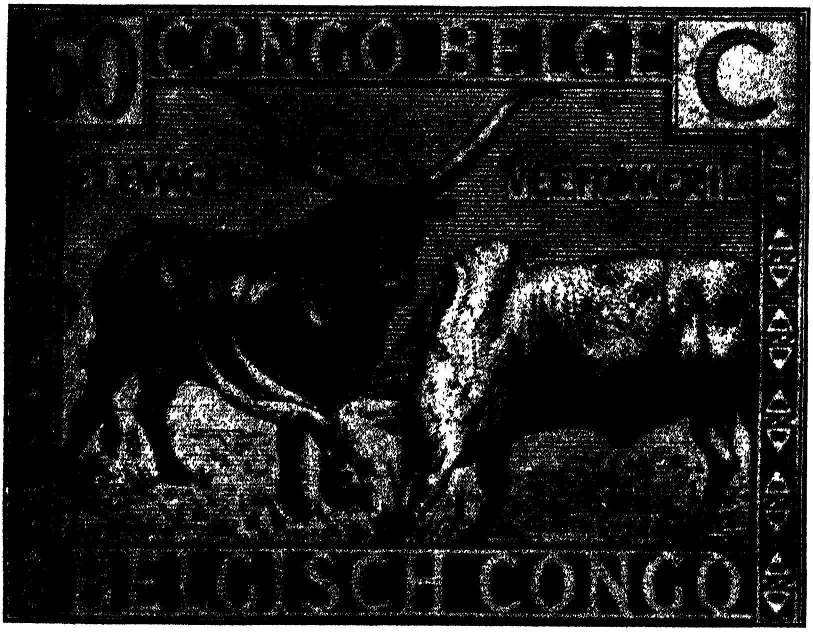

Fig. 54. Cattle from Ruanda-Urundi of Sanga type. Photo of 60 c. red postage stamp of Belgian Congo issued 1926. Note giant horns. It will be observed that the hump of the beast on the right is prominent, but this is not the case in Sanga cattle when grazing. This appearance of the hump is seen only in Zebus with a thoracic hump.

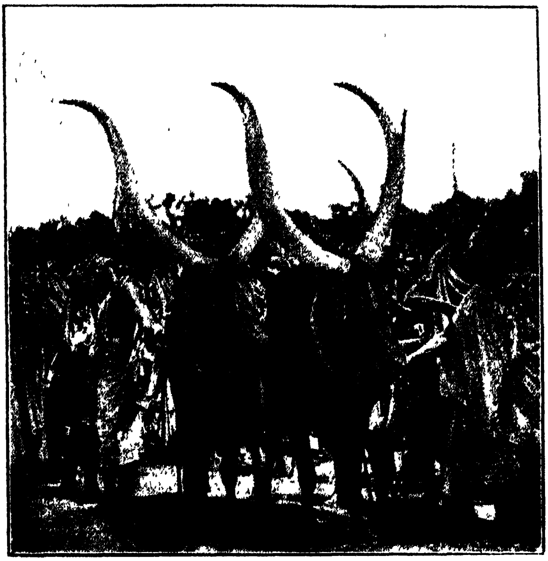

Fig. 55 Another view of Ruanda-Urundi cattle, the so-called sacred cattle "du troupeau des Nyembos du roi du Ruanda". From Fig. 1 frontispiece of Le Plae's work.

TANGANYIKA.

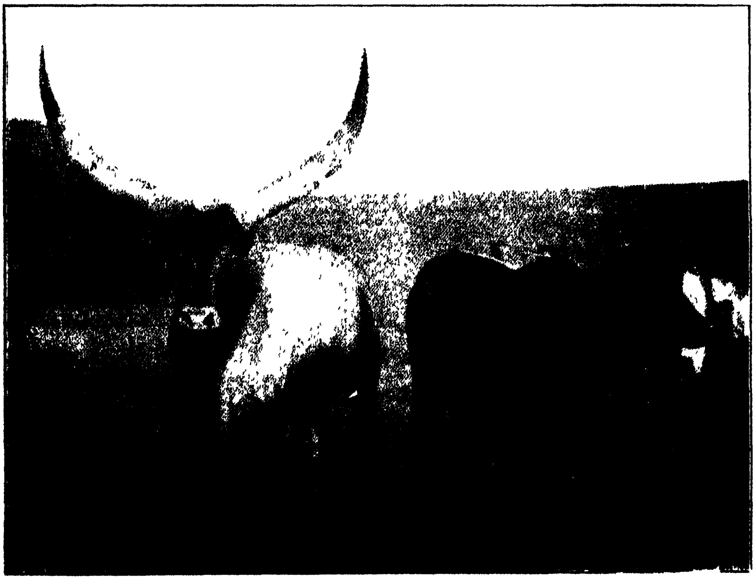

Fig. 56. Ankole (Sanga) bull from Bukoba. Top of shoulder, 52 in.; girth, 68 in.; weight, 850 lb. (F. J. McCall, M.R.C.V.S. *Ann. Rpt. for* 1926.)

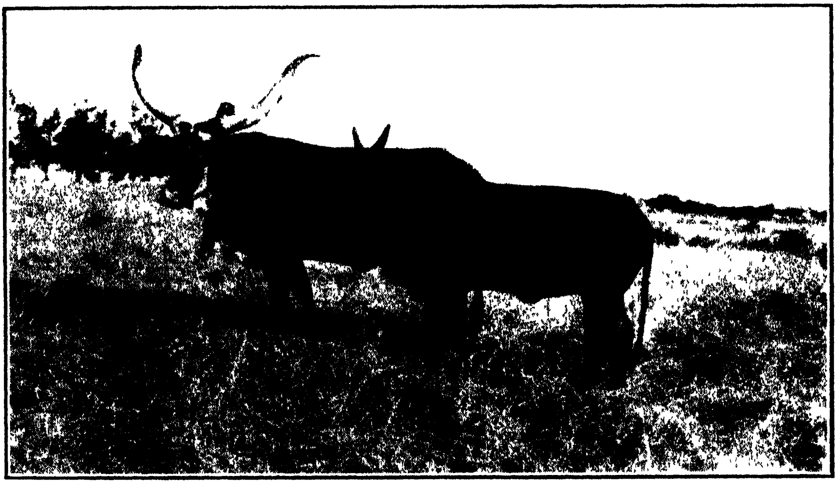

Fig. 57. Ankole (Sanga) cow and calf under good management. (H. E. Hornby, F.R.C.V.S.—his L/18/7 of 2.8.35.)

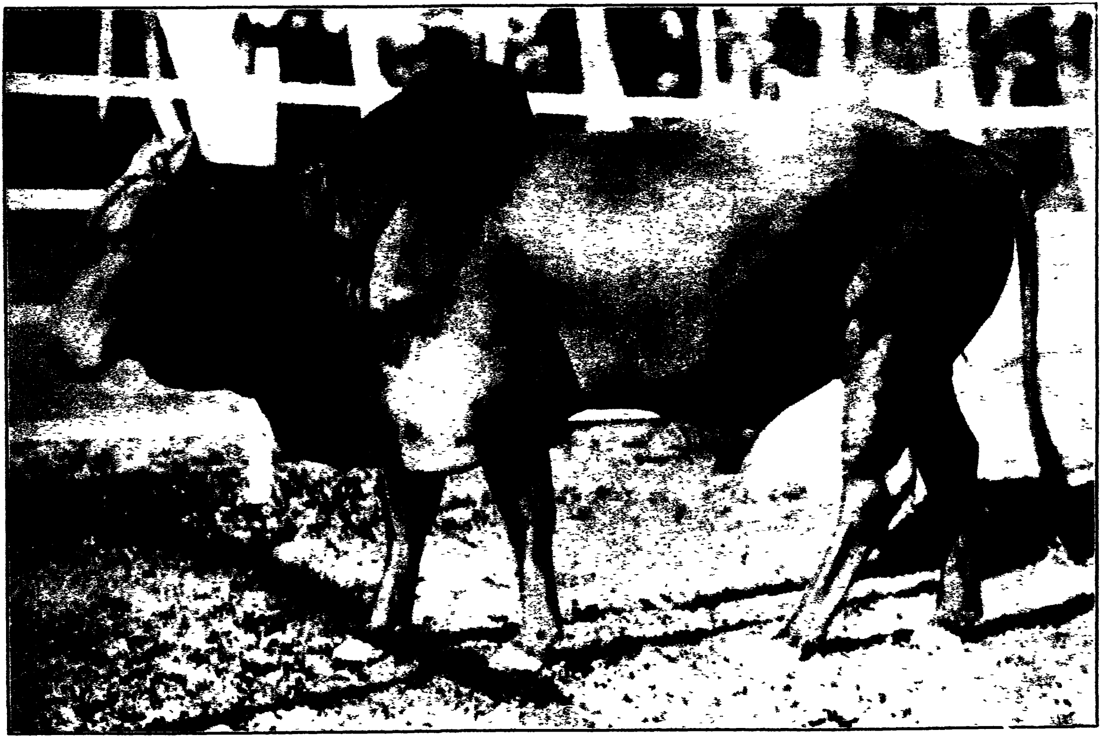

Fig. 58. Shorthorned Zebu (Golden Dun Mkalama) bull, Pugu Road, 2 years. Behind shoulder, 43½ in.; girth, 65 in., weight, 600 lb. (F. J. McCall, M.R.C.V.S. *Ann. Rpt. for* 1926.)

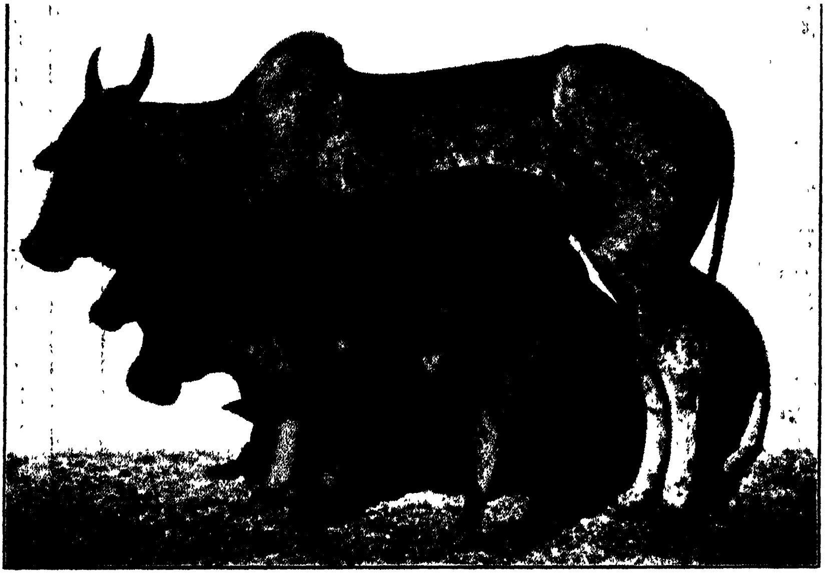

Fig. 59. Shorthorned Zebu (Masai Ugogo) cow and calf, Pugu Road. Height at top of hump, 46 in.; height behind hump, 42 in.; girth, 61½ in. (F. J. McCall, M.R.C.V.S. *Id.*)

NORTHERN RHODESIA.(3)

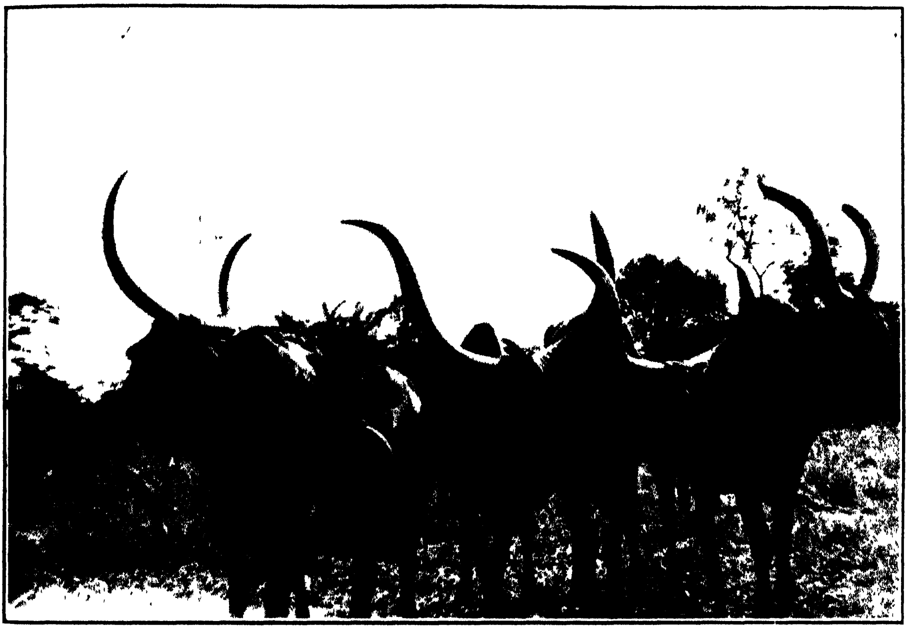

Fig. 60. Barotse (Sanga) oxen from Zambesi Valley. Note cervico-thoracic hump as well as giant horns. (H. S. Purchase, M.R.C.V.S.)

Fig. 61. Baila (Sanga) cow from Mazabuka. Note sturdy build. (R. A. S. McDonald, M.R.C.V.S.)

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(3) For cattle of Bechuana sub-type see Figs. 67-70.

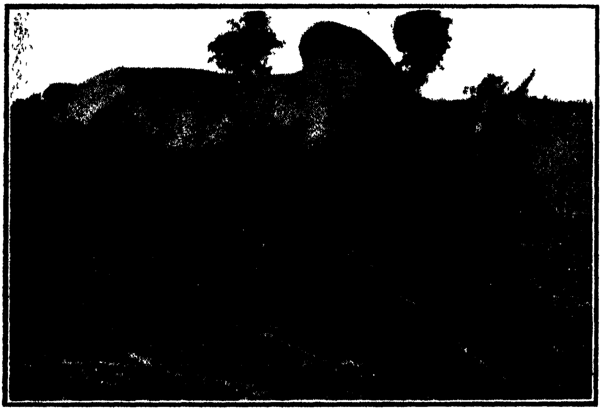


Fig. 62. Shorthorned Zebu (Angoni) cow from Fort Jameson. Note lyre-horns which are not common in East Africa as compared with West Africa. (K. T. Nilsen, B.V.Sc.)

NYASALAND.

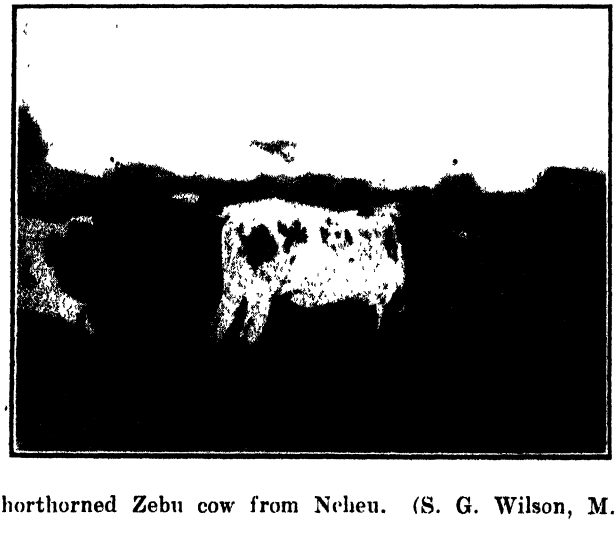


Fig. 63. Shorthorned Zebu cow from Neheu. (S. G. Wilson, M.R.C.V.S.)

SOUTH WEST AFRICA PROTECTORATE.

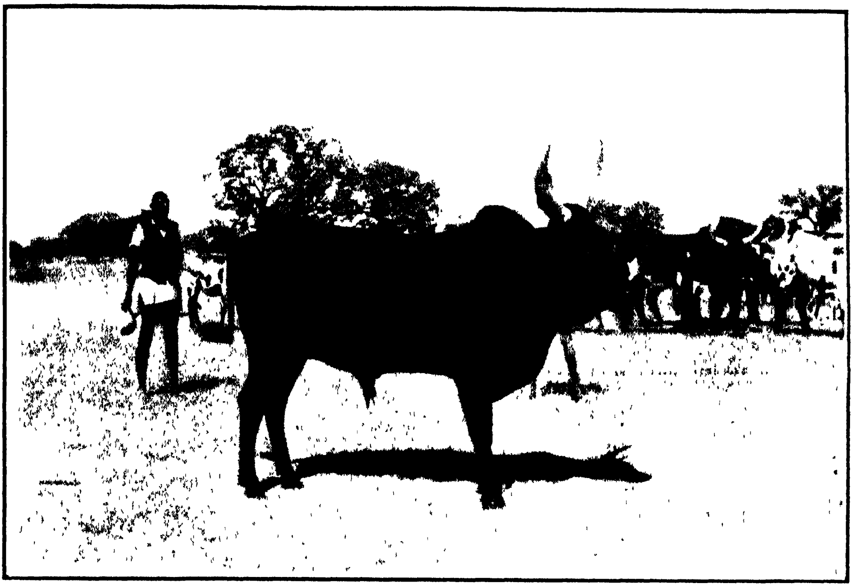


Fig. 64.* Ovambo (Sanga) bull.

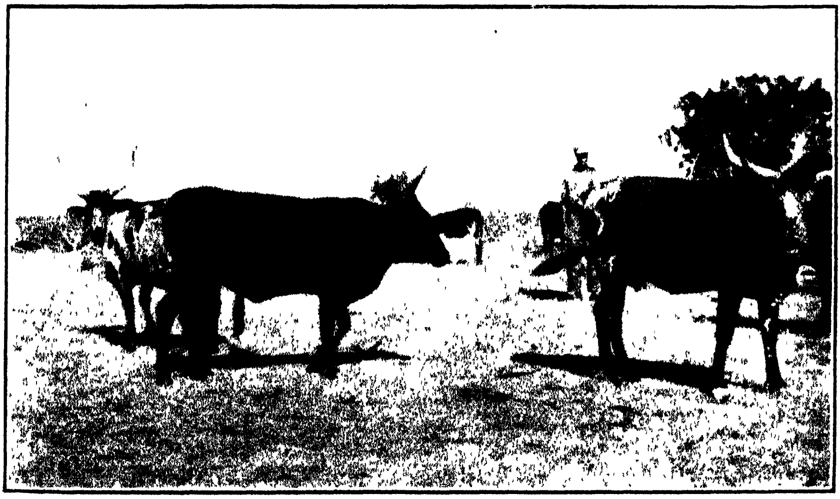


Fig. 65.* Ovambo (Sanga) cows.

Fig. 66. Ovambo (Sanga) cows. (T. Meyer.)

Bechuanaland Protectorate.

Fig. 67. **Bechuana-Batawana (Sanga) bull, near Maun. (H. H. Curson.)**

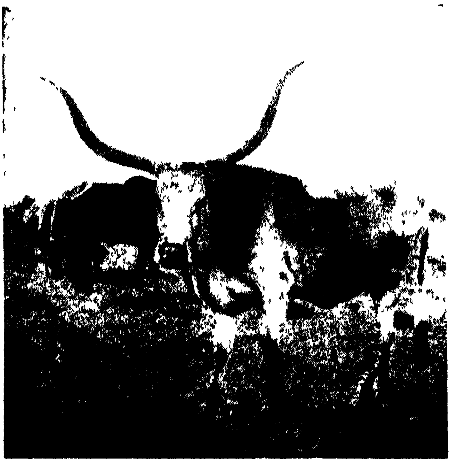


Fig. 68. Bechuana-Batawna-(Sanga) cow, red roan, west of Taoge River, near Namaseri. Horns are of Ankole type. (H. H. Curson.)

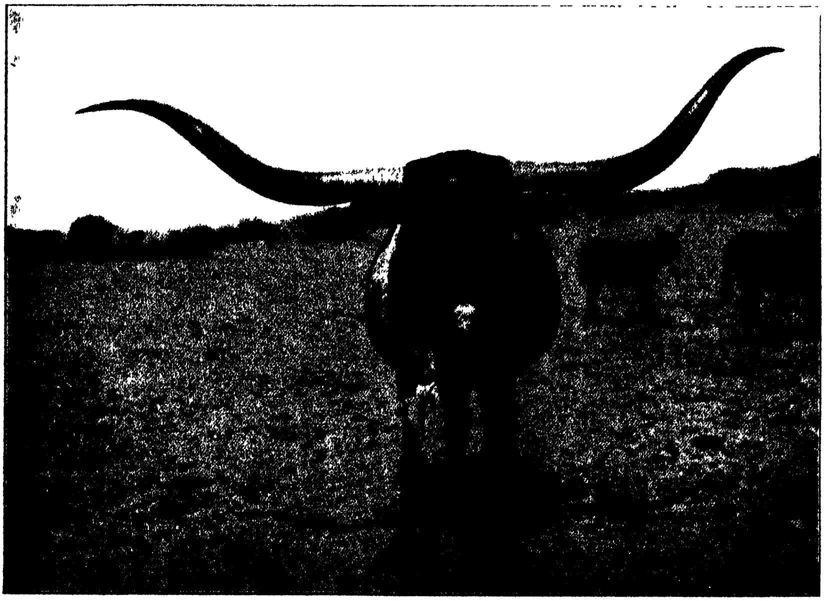


Fig. 69. Bechuana-Batawana-(Sanga) ox with horns directed laterally as in Afrikander. From tip to tip along horns and across head 10 feet. From tip to tip across 8 ft. 6 in. (*Star*, Johannesburg, 8.6.1935.)

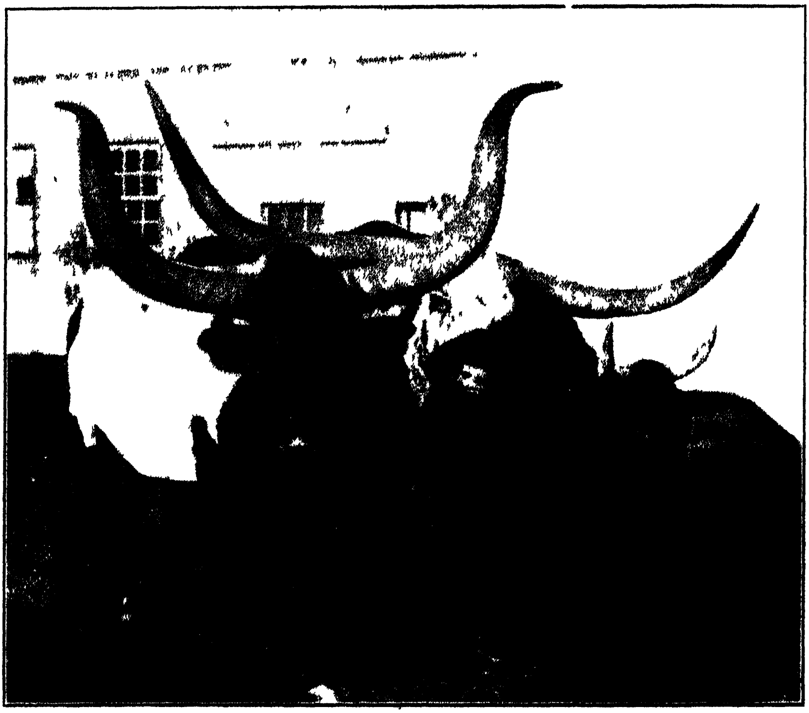


Fig 70. Bechuana-Batawana-(Sanga) oxen and bull. (Miss Wilman, McGregor Museum, Kimberley.)

Fig 71. Ngamiland export trade to Northern Rhodesia. Batawana cattle being taken across Zambesi River at Kazungulu, February, 1931. (H. H. Curson.)

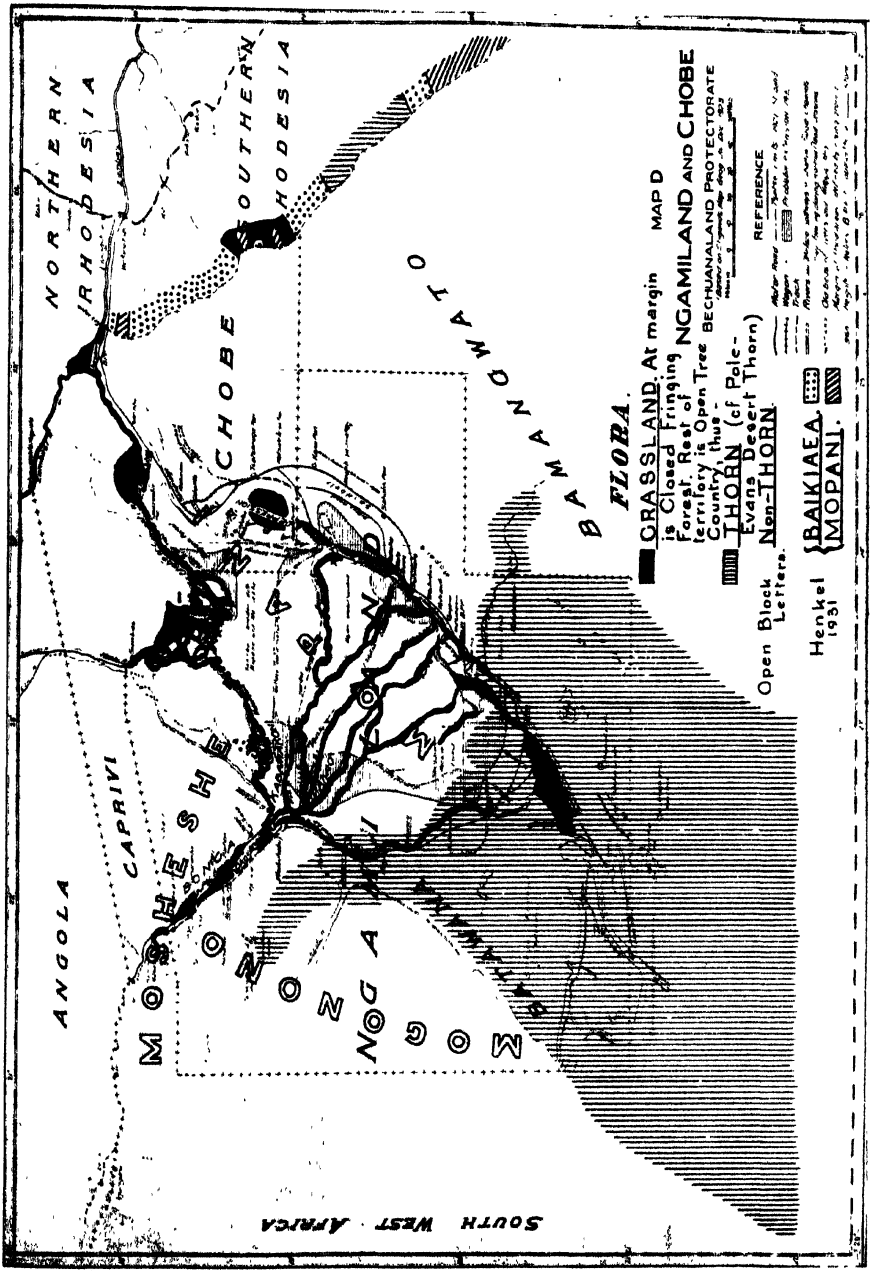


Fig. 72. Map of Ngamiland and Chobe Districts of Bechuanaland Protectorate.

SOUTHERN RHODESIA

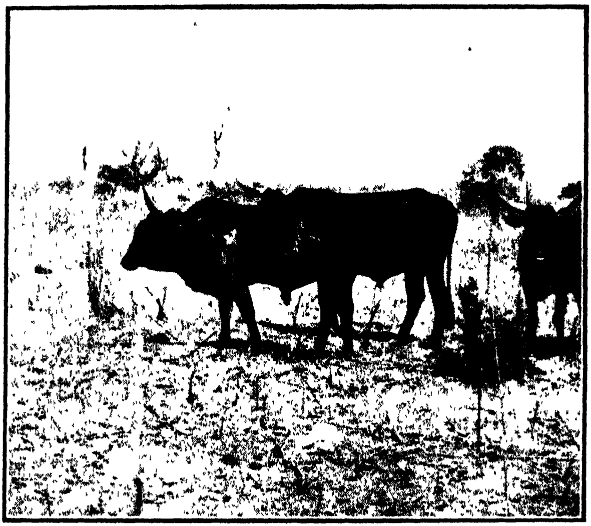


Fig. 73. Makalanga (Sanga) bull and ox, Buhera District. (J H. Badenhorst.)

Fig. 74. Makalanga (Sanga) cow, black, taken at Mr. Goosen's farm near Messina, Transvaal, Easter, 1930. (H. H. Curson.)

MOZAMBIQUE.

Fig. 75. Sanga bull from Magude, near Lourenco Marques. (Dr. C. Sheppard Cruz). Dr. Sheppard Cruz makes the following comment: "Note compact body and short and thick limbs, hump absolutely similar to that of the bulls of the Italian Faenza breed, which belongs also to the *Bos taurus asiaticus*, that is to say, not so pendulous and detached as that of the Zebu bull or that of the Afrikander bull; its dewlap of medium size, and quite different to that of the Afrikander or Zebu; its profile relatively rectilineal (in all the breeds masculinity tends to make the front line more convex, even in animals with a rectilineal profile); the horns are the same type as those of the cow, but shorter and thicker. The cranium is square and the face short."

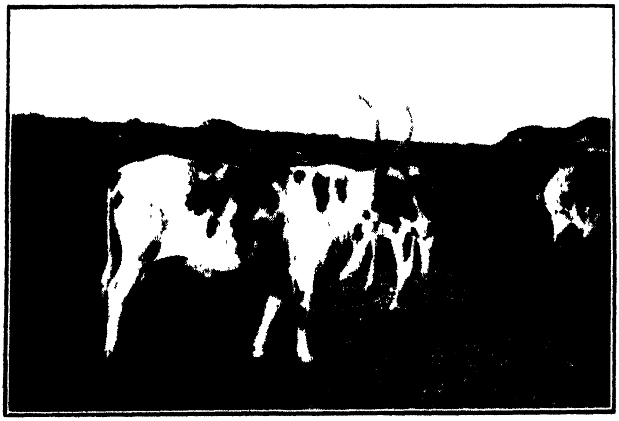


Fig. 76. Sanga cow from Magude, near Lourenco Marques. (Dr. C. Sheppard Cruz). The classification followed in this paper is not necessarily accepted by others, *e.g.* Dr. Sheppard Cruz's remarks on this photo are "doubtless a specimen of the *Bos taurus asiaticus* or *Bos desertorum* (Fitzinger) or "Steppenrind (Brehm)". The forehead is flat and the profile is actually concave.

UNION OF SOUTH AFRICA.

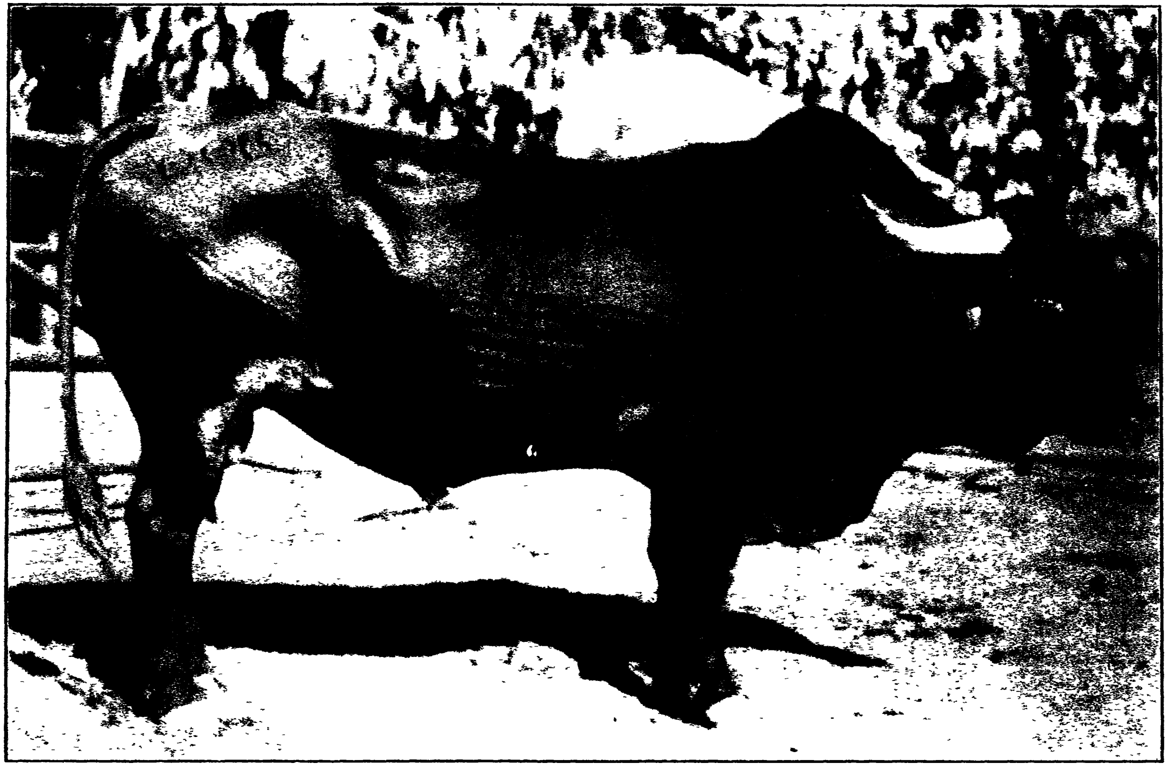


Fig. 77. Afrikander (Lateral-horned Zebu) bull, D.O.B. 4327. Onderstepoort. (T. Meyer.)

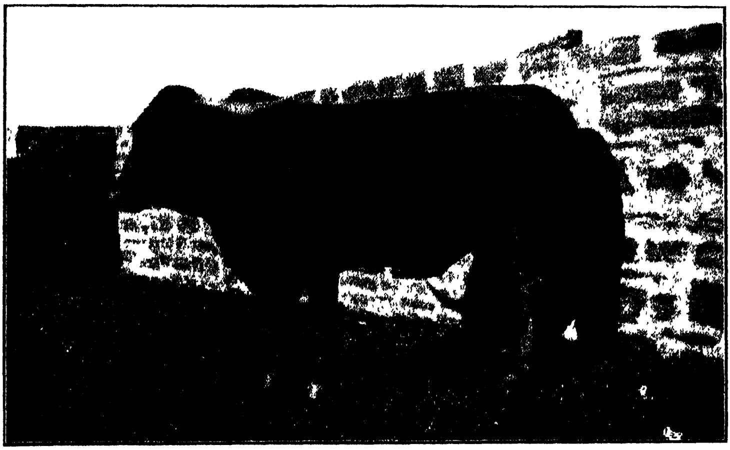


Fig. 78. "Remus" Afrikander cow. University of Pretoria. Age in August, 1929, nearly ten years. Unusually straight in back and somewhat straight in the hocks. (H. H. Curson.)

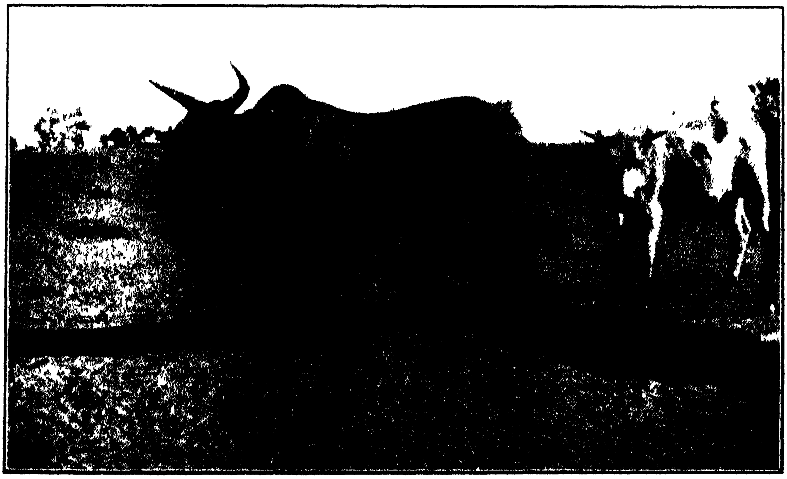


Fig. 79. Zulu (Sanga) bull from Somkele. (H. H. Curson.)

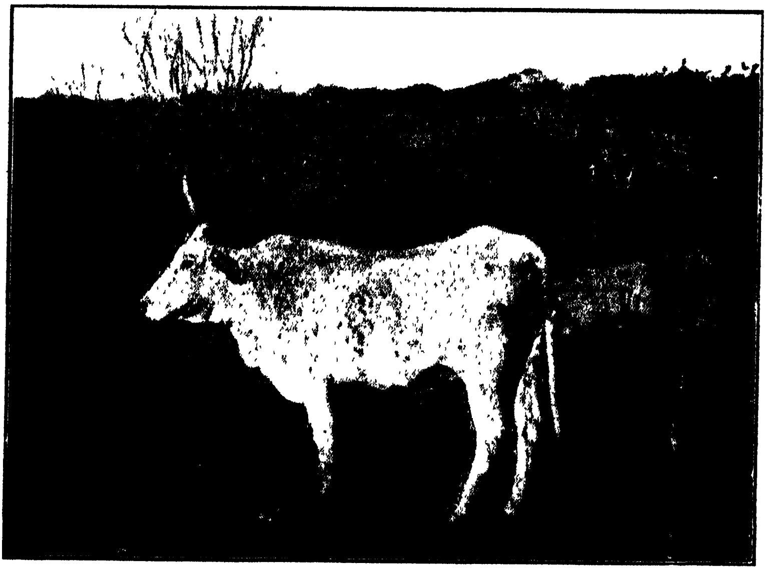


Fig. 80. Zulu (Sanga) cow. The white colour with pigmented skin is not uncommon. These cattle are known to the Zulus as " Nyonmaipumuli ". (J. Papert.)

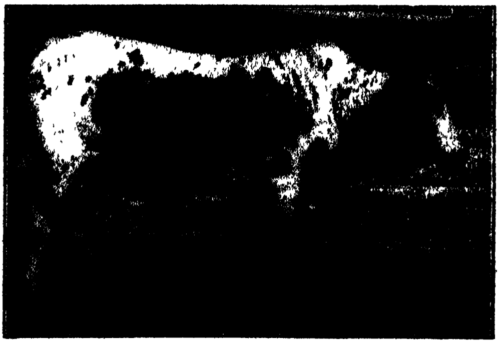


Fig. 81. . Zulu (Sanga) bull. The striking colour is known as Black " Nkone (T. Meyer.)

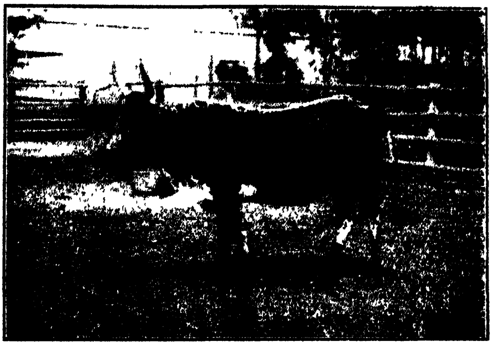


Fig. 82. Zulu (Sanga) cow. (T. Meyer.)

EXTRA AFRICAN CATTLE.

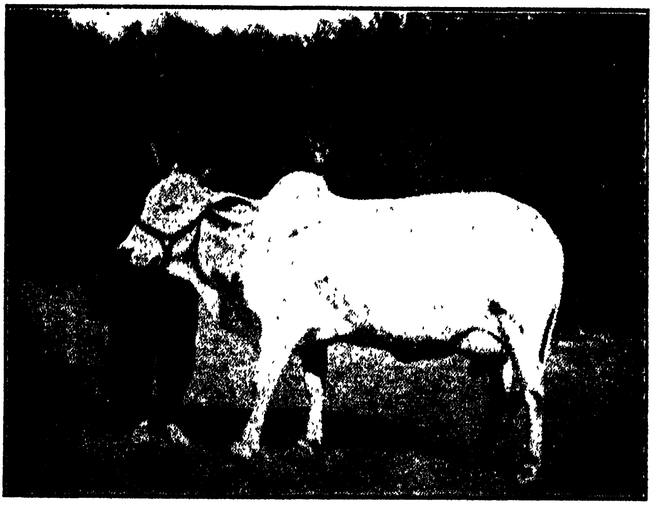


Fig. 83.* Cow of Thar Parkar breed found in South Sindh, Bombay. A good dual purpose beast and resembling the Shorthorned Zebu of East Africa in conformation.

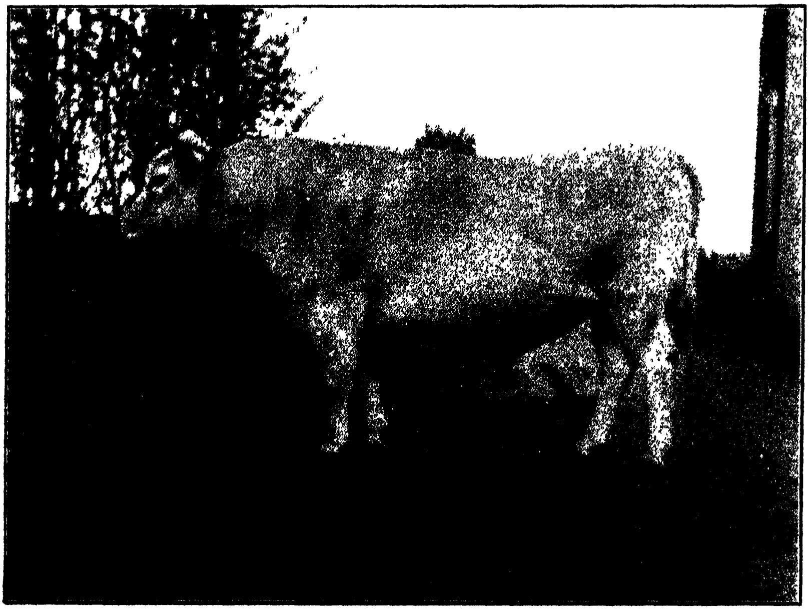


Fig. 84.* Fjallras bull, Sweden. This polled mountain breed although of different type to the "Sidet" cattle of Africa (*e.g.* Zulu and Nigerian) has similar coloration characteristics.

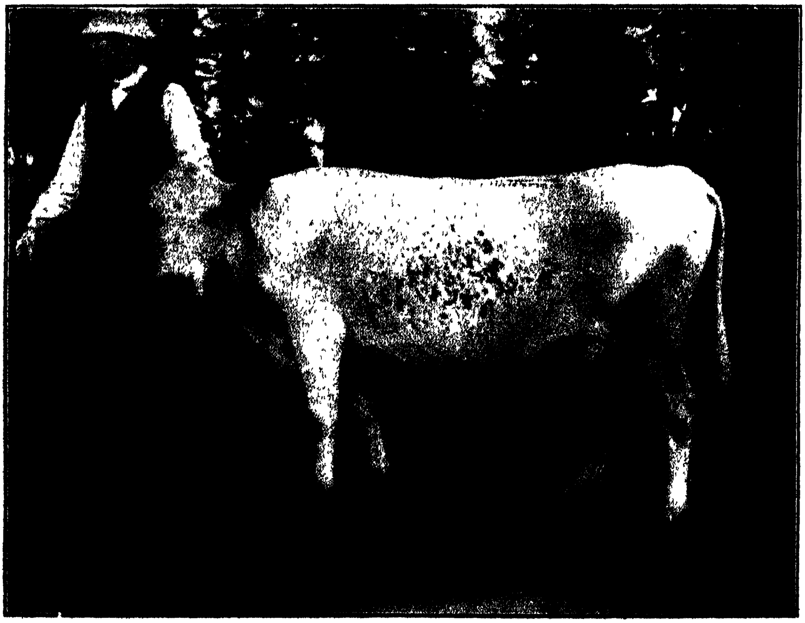


Fig. 85.* Fjallras cow, Sweden. Note the areas of pigmentation.

Fig 86.* Crioulo ox, Argentine. The conformation especially of the skull resembles some of the Sanga sub-types of Africa. The explanation is that both are derived from the original Hamitic Longhorn stock of North Africa. The anterior hump generally seen in Sanga cattle is absent.

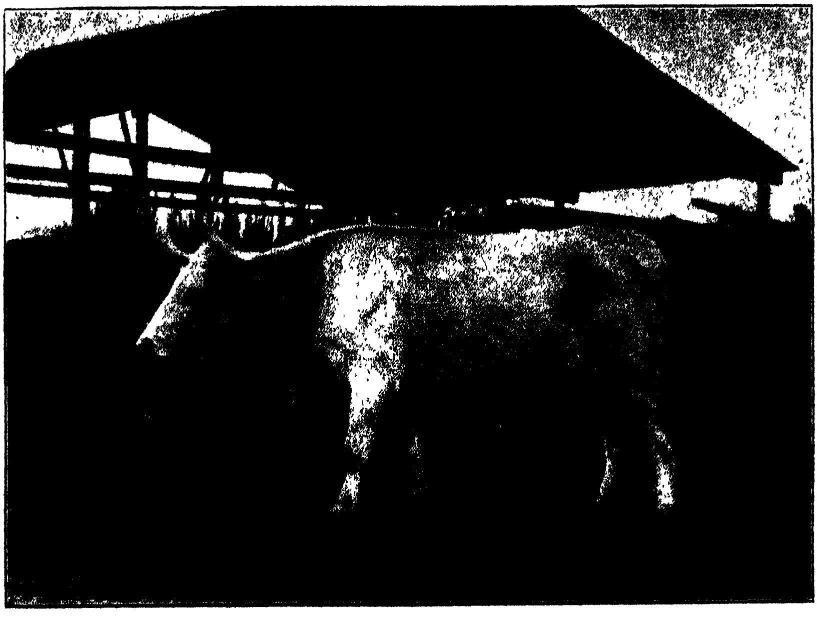
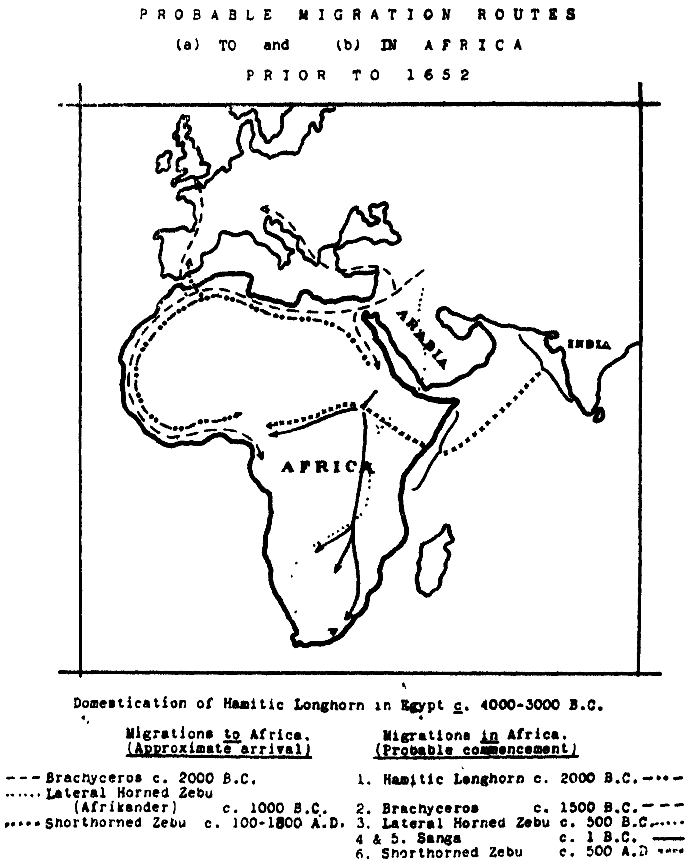
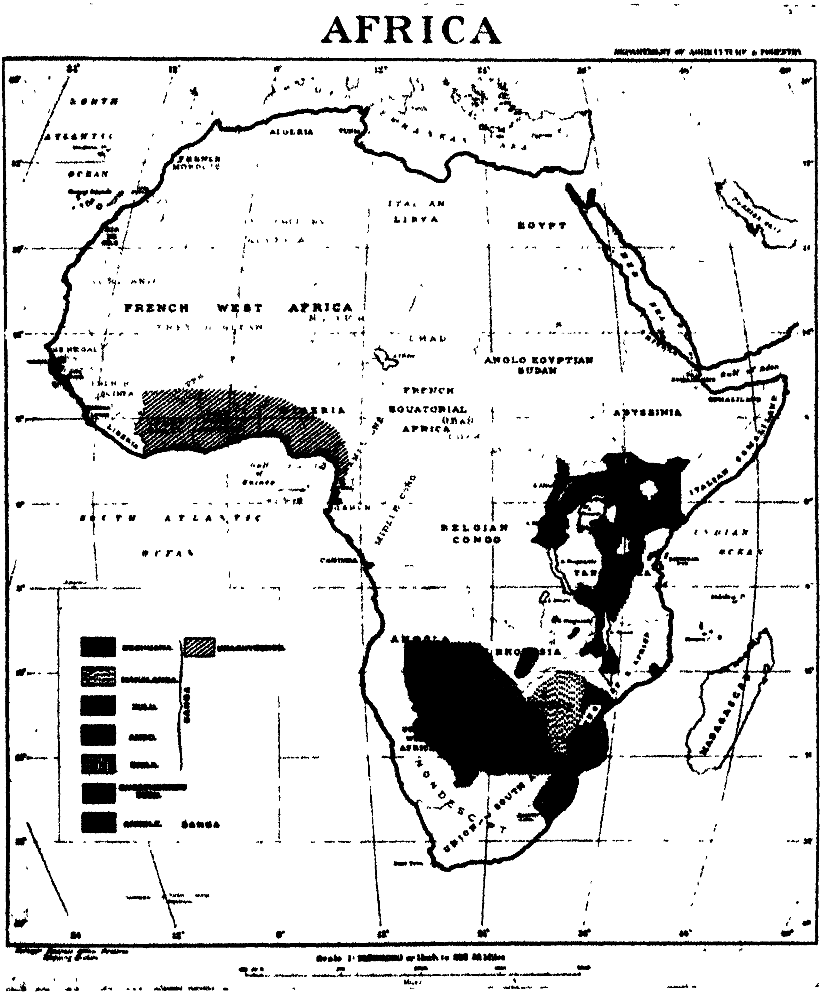


Fig 87.* Another view of a Crioulo ox, Argentine.

MAPS.


PROBABLE MIGRATION ROUTES
(a) TO and (b) IN AFRICA
PRIOR TO 1652
ARABIA
INDIA
AFRICA
Domestication of Hamitic Longhorn in Egypt c. 4000-3000 B.C.
Migrations to Africa. (Approximate arrival)
--- Brachyceros c. 2000 B.C.
...... Lateral Horned Zebu (Afrikander) c. 1000 B.C.
..... Shorthorned Zebu c. 100-1800 A.D.
Migrations in Africa. (Probable commencement)
1. Hamitic Longhorn c. 2000 B.C. -••-
2. Brachyceros c. 1500 B.C. ---
3. Lateral Horned Zebu c. 500 B.C. .....
4 & 5. Sanga c. 1 B.C. ——
6. Shorthorned Zebu c. 500 A.D

1. Probable migration routes (*a*) *to* and (*b*) *in* Africa prior to the European settlement at the Cape of Good Hope (1652).



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